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



OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: October 1, 2008

SUBJECT: ISOXAFLUTOLE: Review of a Developmental Neurotoxicity Study.

PC Code: 123000

Decision No.: 174399

Petition No.: None

Risk Assessment Type: NA

TXR No.: 0050815

MRID No.: 45215701

DP Barcode: D269315

Registration No.: 264-566

Regulatory Action: Section 3

Case No.: NA

CAS No.:

40 CFR: NA

Ver. Apr. 08

FROM: Jess Rowland, Chief
Science Information Management Branch
Health Effects Division (7509P)

And

Robert Mitkus, Ph.D.
Registration Action Branch 1
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THROUGH: Dana Vogel, Chief
Registration Action Branch 1
Health Effects Division (7509P)

TO: Joanne Miller
Registration Manager 23
Registration Division (7508P)

I. CONCLUSIONS:

A developmental neurotoxicity study with Isoxaflutole has been reviewed (MRID 45215701). This study is classified Unacceptable/Guideline and does not satisfy the guideline requirement for a developmental neurotoxicity study in rats. This classification is based on the lack of brain morphometric analyses

II. ACTION REQUESTED:

Review of a developmental neurotoxicity study in rats

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III. BACKGROUND

This study was submitted in response to the conditional registration of this chemical.

IV. RESULTS/DISCUSSION

In a developmental neurotoxicity study (MRID 45215701), isoxaflutole (99.15% a.i.; Lot/Batch # IFT98-196) in 1% methylcellulose was administered by gavage in a volume of 5 mL/kg to pregnant CrI:CD¹(SD)IGS BR rats (25/dose) from GD 6 to LD 10 at doses of 0, 5, 25 or 250 mg/kg/day. P dams were allowed to deliver naturally. All P females were killed on LD 21. On PND 4, eight pups/litter were randomly selected in order to reduce variability among the litters; the remaining offspring were weighed and euthanized. Following weaning, the F₁ offspring remained together as a litter through PND 28 or 29. Subsequently, ten pups/sex/group were selected for neurobehavioral testing and neuropathological examination. Pups not selected for behavioral and neuropathological evaluations were sacrificed on PND 28 or 29. Morphometric analyses, as required by the Guideline, were not performed on offspring (PND 11 or 72) since the evaluation of brains by light microscopy did not reveal any structural abnormalities, nor were there any clear functional differences between the control or treated groups.

No unscheduled parental deaths occurred during the study. Clinical signs, gross pathology, pregnancy rate, number of implantations/dam, gestation length, and sex ratio were unaffected by treatment. Reproductive function was not evaluated. No treatment-related differences in live litter size, post-natal survival, sex ratios, clinical signs, or sexual maturation were observed in any treated group. Body weights of dams at the 5 and 25 mg/kg/day were comparable to that of the controls. In the 250 mg/kg dams, body weights were slightly decreased ($p \leq 0.05$) during GDs 9-15 (decr 4-6%) and LDs 1-10 (decr 7-10%). Body weight gains were decreased ($p \leq 0.01$) during GDs 6-9 (decr 91%) and 9-12 (decr 39%). Body weight gains were also decreased for the entire gestation treatment interval (GDs 6-20; decr 14%, $p \leq 0.01$) and overall gestation (GDs 0-20; decr 11%, $p \leq 0.05$). During the lactation treatment interval (LDs 1-10), body weight gains were comparable to controls in all treated groups. **The maternal LOAEL is 250 mg/kg/day based on decreased body weights, body weight gains, and food consumption. The maternal NOAEL is 25 mg/kg/day.**

No treatment-related differences in live litter size, post-natal survival, sex ratios, clinical signs, or sexual maturation were observed in any treated group. Pup swimming ability, learning, memory, motor activity, auditory startle response, brain weights and dimensions, and neuropathology were unaffected by the test substance.

Body weights of offspring at the 5 and 25 mg/kg/day groups were comparable to that of the controls. In the 250 mg/kg pups, body weights were decreased ($p \leq 0.05$) in the males from PND 1 to 28 (decr 7-12%) and from PND 49 to 63 (decr 4-5%). In the females, body weights were decreased ($p \leq 0.05$) sporadically between PND 1 and 35 (decr 5-12%). Decreased ($p \leq 0.05$) body weight gains were observed sporadically in the males between PNDs 1 and 28 (decr 6-21%) and only during PNDs 4-7 in the females (decr 15%). Absolute brain weights were also reduced (11-12%) in males and females on PND 11 at 250 mg/kg/day, an effect likely related to the decreased body weights and body weight gains observed in these animals.

A NOAEL/LOAEL for offspring can not be determined due to the lack of morphometric analyses in this study.

This study is classified as Unacceptable Guideline due to the lack of morphometric analyses and therefore does not satisfy the guideline requirement (OPPTS 870.6300; OECD 426) for a developmental neurotoxicity study in rats.

ISOXAFLUTOLE (IFT)/123000

OPPTS 870.6300/ OECD 426

EPA Reviewer: Robert Mitkus, PhD

Signature: [Handwritten Signature]
Date: 10/14/08

Registration Action Branch 1, Health Effects Division (7509C)

EPA Secondary Reviewer: Jess Rowland, MS

Signature: Jess Rowland

Chair, DNT Work Group, Health Effects Division (7509C)

Date: 10/14/08

Template version 11/01

TXR#: 0050815

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 123000

DP BARCODE: D269315

SUBMISSION NO.: S585885

TEST MATERIAL (PURITY): Isoxaflutole (99.15% a.i.)

SYNONYMS: RPA 201772; Methanone,(5-cyclopropyl-4-isoxazolyl)[2-(methylsulfonyl)-4-(trifluoromethyl)Phenyl]-(9CI)

CITATION: Nemec, M.D. (2000) An Oral Developmental Neurotoxicity Study of Isoxaflutole (IFT) in Rats. WIL Research Laboratories, Inc., Ashland, OH. Laboratory Study No.: WIL-21153, September 8, 2000. MRID 45215701. Unpublished.

SPONSOR: Aventis CropScience, 2 T.W. Alexander Drive, Research Triangle Park, NC

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 45215701), isoxaflutole (99.15% a.i.; Lot/Batch # IFT98-196) in 1% methylcellulose was administered by gavage in a volume of 5 mL/kg to pregnant CrI:CD⁷(SD)IGS BR rats (25/dose) from GD 6 to LD 10 at doses of 0, 5, 25 or 250 mg/kg/day. P dams were allowed to deliver naturally. All P females were killed on LD 21. On PND 4, eight pups/litter were randomly selected in order to reduce variability among the litters; the remaining offspring were weighed and euthanized. Following weaning, the F₁ offspring remained together as a litter through PND 28 or 29. Subsequently, ten pups/sex/group were selected for neurobehavioral testing and neuropathological examination. Morphometric analyses were not performed. Pups not selected for behavioral and neuropathological evaluations were sacrificed on PND 28 or 29. Positive control data that validate the procedures and observers of the performing lab to assess motor activity, neurotoxicity and behavioral effects were not provided.

No unscheduled maternal deaths occurred during the study. Clinical signs, gross pathology, pregnancy rate, number of implantations/dam, gestation length, and sex ratio were unaffected by treatment. Reproductive function was not evaluated. No treatment-related differences in live litter size, post-natal survival, sex ratios, clinical signs, or sexual maturation were observed in any treated group.

DATA EVALUATION RECORD

ISOXAFLUTOLE (IFT)

Study Type ('83-6a): Developmental Neurotoxicity Study in the Rat

Work Assignment No. 4-02-190A (Formerly 3-01-111A) (MRID 45215701)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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In the 250 mg/kg dams, body weights were slightly decreased ($p \leq 0.05$) during GDs 9-15 (decr 4-6%) and LDs 1-10 (decr 7-10%). Body weight gains were decreased ($p \leq 0.01$) during GDs 6-9 (decr 91%) and 9-12 (decr 39%). Body weight gains were also decreased for the entire gestation treatment interval (GDs 6-20; decr 14%, $p \leq 0.01$) and overall gestation (GDs 0-20; decr 11%, $p \leq 0.05$).

During the lactation treatment interval (LDs 1-10), body weight gains were comparable to controls in all treated groups. Body weight gains were increased ($p \leq 0.01$) during post-treatment (LDs 10-21; inc 133%), resulting in increased body weight gains during the overall lactation interval (LDs 1-21; inc 46%). Absolute (g/animal/day) food consumption was reduced ($p \leq 0.05$) during GDs 6-15 (decr 9-23%), 6-20 (decr 9%), and 0-20 (decr 9%). Absolute food consumption was also decreased during LDs 1-4 (decr 17%, $p \leq 0.01$). Relative (g/kg/day) food consumption was reduced ($p \leq 0.01$) during the GD intervals 6-9 (decr 19%), 9-12 (decr 14%), and 15-20 (decr 11%). In addition, relative food consumption was increased ($p \leq 0.05$) post-treatment during LDs 10-16 (incr 9%) and 10-21 (incr 6%). No treatment-related findings were observed in dams treated with 25 or 5 mg/kg a.i.

The maternal LOAEL is 250 mg/kg/day based on decreased body weights, body weight gains, and food consumption. The maternal NOAEL is 25 mg/kg/day.

No treatment-related differences in live litter size, post-natal survival, sex ratios, clinical signs, or sexual maturation were observed in any treated group. Pup swimming ability, learning, memory, motor activity, auditory startle response, brain weights and dimensions, and neuropathology were unaffected by the test substance.

In the 250 mg/kg pups, body weights were decreased ($p \leq 0.05$) in the males from PND 1 to 28 (decr 7-12%) and from PND 49 to 63 (decr 4-5%). In the females, body weights were decreased ($p \leq 0.05$) sporadically between PND 1 and 35 (decr 5-12%). Decreased ($p \leq 0.05$) body weight gains were observed sporadically in the males between PNDs 1 and 28 (decr 6-21%) and only during PNDs 4-7 in the females (decr 15%). Absolute brain weights were also reduced (11-12%) in males and females on PND 11 at 250 mg/kg/day, an effect likely related to the decreased body weights and body weight gains observed in these animals. Body weights of offspring at the 5 and 25 mg/kg/day groups were comparable to that of the controls.

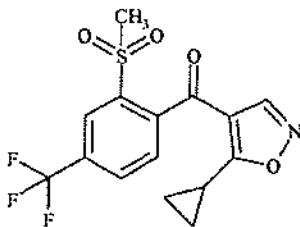
A NOAEL/LOAEL for offspring cannot be determined due to the lack of morphometric analyses in this study.

This study is classified as **Unacceptable/Guideline** due to the lack of morphometric analyses and therefore does not satisfy the guideline requirement (OPPTS 870.6300; OECD 426) for a developmental neurotoxicity study in rats

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

ISOXAFLUTOLE (IFT)/123000**I. MATERIALS AND METHODS****A. MATERIALS**

- 1. Test Material:** Isoxaflutole technical
- Description:** Fine, off-white powder
- Lot/Batch #:** IFT98-196
- Purity:** 99.15 % a.i.
- Compound Stability:** The test substance was stable in the vehicle for 8 days at room temperature.
- CAS # of TGA1:** 141112-29-0



- 2. Vehicle and/or positive control:** 1% methylcellulose in deionized water

3. Test animals (P)

Species:	Rat
Strain:	Crj:CD®(SD)IGS BR
Age at study initiation:	Approximately 13 weeks
Wt. at study initiation:	286-294 g
Source:	Charles River Laboratories, Inc. Raleigh, NC
Housing:	Following successful mating, females were housed individually in plastic maternity cages with nesting material. The females and offspring were housed together in these cages through lactation day (LD) 21. Following weaning, the F1 offspring remained together as a litter through PND 28 or 29. Subsequently, offspring selected for evaluation of developmental landmarks, neurobehavioral testing, and neuropathological examination were housed individually in wire mesh cages until their scheduled termination.
Diet:	Certified Rodent LabDiet® 5002 (PMI Nutrition International), <i>ad libitum</i>
Water:	Reverse osmosis treated tap water, <i>ad libitum</i>
Environmental conditions:	Temperature: 71.8-72.9°F Humidity: 40.8-66.9% Air changes: Approximately 10/hour Photoperiod: 12 hrs dark/12 hrs light
Acclimation period:	14 days

B. PROCEDURES AND STUDY DESIGN

- 1. In life dates** - Start: 7/26/99 End: 8/2/00
- 2. Study schedule:** P females were administered the test substance daily via gavage from GD 6 until LD 10. Females were allowed to deliver naturally. All P females (including those that did not deliver) were killed on LD 21. On post-natal day (PND) 4, eight pups/litter were randomly selected in order to reduce variability among the litters; the remaining offspring were weighed and euthanized. Following weaning, the F₁ offspring remained together as a litter through PND 28 or 29. All pups were evaluated for either vaginal patency or balanopreputial separation.

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Subsequently, ten pups/sex/group were selected for evaluation of neurobehavioral testing, and neuropathological examination. Pups not selected for behavioral evaluations were sacrificed on PND 28 or 29.

3. **Mating procedure:** At approximately 84 days of age, females were paired with males (1:1) for mating. Successful mating was determined by the presence of a copulatory plug or sperm in a vaginal smear. The day on which successful mating was determined was designated as gestation day (GD) 0. Following successful mating, females were housed individually in plastic maternity cages with nesting material. The females and offspring were housed together in these cages through lactation day (LD) 21.
4. **Animal Assignment:** Mated females were randomly assigned (stratified by body weight) to test groups as shown in Table 1. Dams were assigned to functional observation testing as shown.

Offspring were assigned to testing subgroups at the time of litter standardization on PND 4 (Table 1).

Table 1. Study design. *

Experimental Parameter	Dose (mg/kg/day)			
	0	5	25	250
Maternal Animals				
No. of maternal animals assigned	25	25	25	25
FOB (GDs 6, 12; LDs 4, 7)	10	10	10	10
Offspring				
Detailed clinical/FOB (PND 4, 11, 21, 35, 45, 60)	10/sex	10/sex	10/sex	10/sex
Motor activity (PND 13, 17, 21, 61±2 days)	10/sex	10/sex	10/sex	10/sex
Auditory startle habituation (PND 20, 60±2 days)	10/sex	10/sex	10/sex	10/sex
Learning and memory (PND 22, 62)	10/sex	10/sex	10/sex	10/sex
Brain weight				
PND 11	10/sex	10/sex	10/sex	10/sex
PND 72 ^b	10/sex	10/sex	10/sex	10/sex
Neuropathology				
PND 11	10/sex	10/sex	10/sex	10/sex
PND 72 ^b	10/sex	10/sex	10/sex	10/sex

a Data obtained from Study Report pages 27, 35, 38, 40, 41, and 42.

b Animals were randomly selected from those pups dedicated to motor activity, auditory startle, and learning and memory tests.

5. **Dose selection rationale:** No dose selection rationale was provided.
6. **Dosage administration:** Doses were administered daily via gavage at 5 mL/kg from GD 6 through LD 10. Dosing was based on the most recent body weight determination.
7. **Dosage preparation and analysis:** Dose formulations were prepared weekly by weighing the test substance and diluting it to the desired concentrations with 1% methylcellulose. The dose formulations were stored at room temperature and stirred continuously during use. Homogeneity and stability were determined prior to the initiation of dosing on representative batches of 1, 5,

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and 50 mg/mL dose formulations. Samples (top, middle, bottom) of each dose formulation were analyzed for homogeneity or stored at room temperature for eight days and analyzed for stability. Concentration was determined for each dose formulation prepared weekly during the study.

Results -

Homogeneity (range as mean % of nominal): 109-112%

Stability (range as mean % of day 0): 95.8-97.8%

Concentration: Acceptable concentrations ranged from 93-110% of nominal; however, this range excludes one analysis of a 50 mg/mL formulation (53% of nominal). Reanalysis of this formulation confirmed the low concentration (74% of nominal). The Sponsor stated that analysis of a sample from a daily aliquot from this formulation was within acceptable limits, and that the impact to the study was minimal. It should be noted that animals were dosed with the questionable dosing formulation for two days.

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS**1. In-life observations**

- a. **Maternal animals:** The P animals were checked for mortality twice daily; clinical signs were recorded daily from GD 0 through LD 21. In addition, animals were monitored for clinical signs of toxicity for approximately one hour post-dosing throughout the treatment interval. Dams that delivered were evaluated three times daily during the period of expected parturition. Body weights and food consumption were measured on GDs 0, 3, 6, 9, 12, 15, and 20 and on LDs 1, 4, 7, 10, 16, and 21. Body weight gains were calculated for each corresponding gestation or lactation interval, as well as for GDs 6-20 and 0-20 and LDs 1-10, 10-21, and 1-21.

Ten dams/group were randomly selected for observations outside of the home cage on GDs 6 and 12 and LDs 4 and 7. Females that failed to deliver were replaced by randomly selected dams that did deliver. Testing was performed by the same trained technicians, when possible, without knowledge of the treatment status of the animals. The following parameters were evaluated:

FUNCTIONAL OBSERVATIONS	
Ease of removal from cage	Salivation
Lacrimation/chromodacryorrhea	Fur appearance
Piloerection	Respiratory rate/character
Palpebral closure	Mucous membranes/eye/skin color
Red/crusty deposits	Muscle tone
Eye prominence	Gait
Mobility	Arousal
Convulsions/tremors	Urination/defecation
Grooming	Backing
Bizarre/stereotypic behavior	
Ease of handling	

P females that did not deliver a litter were sacrificed on presumed GD 25, necropsied, and examined for gross lesions and evidence of pregnancy. All other P dams were sacrificed on LD 21 and subjected to a gross pathological examination. Tissues were preserved in 10% neutral-buffered formalin for possible future histopathological examination.

b. Offspring

- 1) **Litter observations:** Pups were evaluated daily for mortality and morbidity. Clinical observations and body weights were recorded on PNDs 1, 4, 7, 11, 14, 17, and 21, and then weekly thereafter until sacrifice. Post-weaning food consumption was not reported. The following additional litter observations (X) were made (Table 2):

Table 2. Litter observations.^a

Observation	Post-natal Day							
	0	1	4	7	11	14	17	21
Number of live pups ^b	X	X	X	X	X	X	X	X
Pup weight		X	X	X	X	X	X	X
Clinical observations		X	X	X	X	X	X	X
Number of dead pups ^b	X	X	X	X	X	X	X	X
Sex of each pup	X		X		X			X

^a Data obtained from the study report, pages 34-36.

^b Observed daily.

On PND 4, litters were standardized by randomly selecting 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were weighed, killed, and discarded. It was stated that litters that did not meet the sex ratio criteria (M/F ratios of 4:4, 5:3, or 3:5) were not to be used for neurobehavioral or neuropathological evaluations and were to be killed and necropsied on PND 4. However, the litter from one 25 mg/kg dam (No. 25927) had a male/female ratio of 2:6 and the pups were not euthanized on PND 4; furthermore, female pup (No. 9) was selected for brain weights on PND 11, females pups (Nos. 2, 5, 6, 7, and 10) were euthanized on PND 28, and males pups (Nos. 1 and 3) were euthanized on PND 72 (not selected for brain weights).

- 2) **Developmental landmarks:** Each remaining male pup was observed for balanopreputial separation beginning on PND 35; daily examinations continued until balanopreputial separation was observed. Each remaining female pup was observed daily for vaginal patency beginning on PND 25 and continuing until vaginal patency was observed. Additionally, body weights were recorded on the day that vaginal opening or preputial separation was achieved.
- 3) **Postweaning observations:** After weaning on PND 21, offspring were examined daily for mortality and morbidity. Clinical observations and body weights were recorded weekly until sacrifice.
- 4) **Neurobehavioral evaluations:** Observations and the schedule for those observations are summarized as follows from the report.
 - i) **Functional observational battery (FOB):** On postnatal days 4, 11, 21, 35, 45, and 60, a total of 10 offspring/sex/group were examined outside the home cage in an FOB assessment, as appropriate for the developmental stage being observed. The same parameters assessed in the maternal FOB were examined, with the following exceptions on PND 4 and 11: piloerection, fur appearance, palpebral closure, eye color, eye prominence, gait, backing, and grooming were not recorded because of the eyes not being open, incomplete fur growth, or inability to complete sufficient movement to determine gait, backing, or grooming.
 - ii) **Motor activity testing:** Motor activity measurements were performed on 10 pups/sex/group on PNDs 13, 17, 21, and 61 (± 2 days) using an automatic monitoring device (SDI Photobeam Activity System, San Diego Instruments, San Diego, CA). Data were collected in five-minute intervals over the course of 60 minutes. Ambulatory (interruption of two or more consecutive photobeams) and total (fine motor skills, i.e., interruption of a single photobeam plus ambulatory motor activity) motor activity were evaluated.
 - iii) **Auditory startle reflex habituation:** Acoustic startle response was evaluated for 10 pups/sex/group on PNDs 20 and 60 (± 2 days) using the SR-Lab Startle Response System (San Diego Instruments, San Diego, CA). Each test session consisted of 50 trials, with an 8 second inter-trial interval; startle response data were analyzed in five blocks of 10 trials each. Measurements included maximum response amplitude (V_{max}), average response amplitude (V_{ave}) and latency to V_{max} (T_{max}).
 - iv) **Learning and memory testing:** Swimming ability and learning and memory were evaluated for 10 pups/sex/group beginning on PND 22 or 62 (animals used for the PND 22 interval were not used for the PND 62 interval). A water-filled, eight unit T-maze (similar to Biel) was utilized for testing. Each testing interval consisted of three phases conducted over seven consecutive days. Phase 1, conducted on Day 1 of testing, evaluated swimming ability and motivation to escape the maze; each animal was allowed four trials. Phase 2, conducted on Days 2-6 of testing, evaluated sequential learning and allowed each animal two trials/day for 2-3 consecutive days to navigate two separate mazes within 3 minutes. Phase 3, conducted on Day 7 of testing, evaluated memory by challenging the animal to solve one of the mazes from Phase 2 in two trials. Biel maze data were evaluated as the mean time to escape over all trials for each of the three phases. In addition,

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the number of errors were tabulated for Phases 2 and 3.

2. Postmortem observations

- a. **Maternal animals:** Maternal animals were sacrificed via CO₂ asphyxiation on LD 21 and subjected to gross necropsy. The number of former implantation sites was recorded and tissues were preserved in 10% neutral buffered formalin for possible future histopathological examination.
- b. **Offspring:** The offspring selected for brain weight or neuropathological evaluation were sacrificed on PND 11 or 72. These animals were subjected to postmortem examinations as described below.

On PND 11, 10 pups/sex/group (1 pup/sex/litter) were sacrificed by perfusion fixation and subjected to neuropathological examination. The brains (with olfactory bulbs) were removed, weighed, and measured. Sections from all major brain regions (including olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain, brainstem, and cerebellum) were trimmed, processed into paraffin blocks, sectioned, and stained with hematoxylin and eosin. Tissues from the control and 250 mg/kg pups were examined microscopically.

On PND 72, 10 pups/sex/group (1 pup/sex/litter) were randomly selected from the pups subjected to motor activity, auditory startle response, and learning and memory tests; euthanized by carbon dioxide inhalation; and perfused *in situ*. The central and peripheral nervous system and tissues were dissected, preserved, embedded in paraffin or plastic, sectioned, and stained with hematoxylin and eosin. The following CHECKED (X) tissues from the control and 250 mg/kg pups were examined microscopically:

CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM	
	BRAIN		SCIATIC NERVE
X	Olfactory bulbs	X	Sciatic Nerves
X	Cerebral cortex		
X	Midbrain		OTHER
X	Cerebellum	X	Sural Nerve
X	Hippocampus	X	Tibial Nerve
X	Medulla oblongata	X	Peroneal Nerve
X	Basal ganglion	X	Lumbar dorsal root ganglion
X	Thalamus	X	Lumbar dorsal root fibers
X	Hypothalamus	X	Lumbar ventral root fibers
	SPINAL CORD	X	Cervical dorsal root ganglion
X	Cervical swelling	X	Cervical dorsal root fibers
X	Lumbar swelling	X	Cervical ventral root fibers
	OTHER		
X	Gasserian Ganglion		
X	Trigeminal nerves		
X	Optic nerves		
X	Eyes		
X	Skeletal muscle		

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The report stated that the evaluation of brains by light microscopy did not reveal any structural abnormalities, nor were there any clear functional differences between the control or treated groups. Therefore, morphometric analyses on PND 11 and 72 were not performed as the sponsor requested.

D. DATA ANALYSIS

1. Statistical analyses: The following statistical procedures were used:

Statistical test ^a	Parameter
ANOVA (two-tailed) with Dunnett's test	Maternal body weights and body weight gains during gestation and lactation Maternal food consumption Mean litter weights Length of gestation Implantation sites Unaccounted sites Number of pups born Live litter size Organ weights Startle response Biel maze Pup weights Day of balanopreputial separation Day of vaginal patency
Multtest procedure	Motor activity
Kruskal-Wallis test with Mann-Whitney U-test	Sex ratio at birth Post-natal survival
Kolmogorov-Smirnov test	Histopathological findings

a Significance was determined at $p \leq 0.05$.

2. Indices: The following indices were calculated by the Sponsor:

Mean live litter size = # viable pups at PND 0 / # litters with viable pups at PND 0

Post-natal survival (%) = $\sum (\# \text{ viable pups per litter at end of interval } N / \# \text{ viable pups per litter at start of interval } N) / \# \text{ litters per group} \times 100$

3. Positive and historical control data: Positive control data that validate the procedures and observers of the performing lab to assess motor activity, neurotoxicity and behavioral effects were not provided. Historical control data were provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical and functional observations: No unscheduled deaths occurred during

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the study. No treatment-related clinical or FOB signs were noted in any group.

2. **Body weight and food consumption:** Body weights and body weight gains for the P females are presented in Tables 2a and 2b. Body weights were slightly decreased ($p \leq 0.05$) in the 250 mg/kg P females during gestation days (GDs) 9-15 ($\downarrow 4-6\%$) and lactation days (LDs) 1-10 ($\downarrow 7-10\%$). Body weight gains were decreased ($p \leq 0.01$) in the 250 mg/kg P females during GDs 6-9 ($\downarrow 91\%$) and 9-12 ($\downarrow 39\%$). Body weight gains were also decreased for the entire gestation treatment interval (GDs 6-20; $\downarrow 14\%$, $p \leq 0.01$) and overall gestation (GDs 0-20; $\downarrow 11\%$, $p \leq 0.05$). During the lactation treatment interval (LDs 1-10), body weight gains were comparable to controls in all treated groups. Body weight gains were increased ($p \leq 0.01$) in the 250 mg/kg P females during post-treatment LDs 10-21 ($\uparrow 133\%$), resulting in increased body weight gains during LDs 1-21 ($\uparrow 46\%$).

Table 2a. Selected mean (\pm S.D.) body weights (g) for P females administered isoxaflutole from GD 6 to LD 10. ^a

Treatment interval (days)	Dose (mg/kg/day)			
	0	5	25	250
Gestation				
0	261 \pm 14.7	258 \pm 11.9	256 \pm 13.4	258 \pm 14.1
6	294 \pm 19.8	292 \pm 11.9	286 \pm 15.4	291 \pm 15.1
9	305 \pm 18.7	304 \pm 13.3	297 \pm 17.9	292* \pm 12.2 ($\downarrow 4$)
15	341 \pm 28.4	334 \pm 21.7	332 \pm 21.3	320** \pm 15.4 ($\downarrow 6$)
20	411 \pm 35.3	408 \pm 20.1	403 \pm 26.6	392 \pm 19.3
Lactation				
1	309 \pm 30.6	305 \pm 22.7	297 \pm 19.4	279** \pm 18.5 ($\downarrow 10$)
4	326 \pm 31.3	321 \pm 20.3	315 \pm 19.5	300** \pm 17.1 ($\downarrow 8$)
10	351 \pm 26.7	344 \pm 20.0	337 \pm 20.4	325** \pm 23.6 ($\downarrow 7$)
21	345 \pm 23.0	341 \pm 19.3	336 \pm 21.2	333 \pm 17.6

a Data were obtained from the study report Tables 6 and 8, pages 94 and 96. Percent difference from controls is presented parenthetically; n= 23-25.

* Significantly different from controls at $p \leq 0.05$.

** Significantly different from controls at $p \leq 0.01$.

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Table 2b. Selected mean (\pm S.D.) body weight gains (g) for P females administered isoxaflutole from GD 6 to LD 10.^a

Treatment interval (days)	Dose (mg/kg/day)			
	0	5	25	250
Gestation				
0-3	18 \pm 4.6	19 \pm 5.8	15 \pm 5.1	18 \pm 6.4
6-9	11 \pm 3.6	12 \pm 3.8	11 \pm 3.6	11** \pm 7.3 (\downarrow 91)
9-12	18 \pm 6.4	17 \pm 3.8	17 \pm 4.1	11** \pm 7.4 (\downarrow 39)
Treatment (6-20)	117 \pm 25.0	116 \pm 13.9	116 \pm 17.4	101** \pm 16.7 (\downarrow 14)
Overall (0-20)	150 \pm 27.6	149 \pm 18.8	146 \pm 19.4	133** \pm 19.1 (\downarrow 11)
Lactation				
1-4	17 \pm 16.2	16 \pm 15.3	18 \pm 11.8	21 \pm 10.7
7-10	19 \pm 10.7	21 \pm 13.3	19 \pm 15.9	18 \pm 15.7
Treatment (1-10)	43 \pm 12.0	40 \pm 17.6	40 \pm 11.9	46 \pm 18.2
Post-treatment (10-21)	-6 \pm 15.2	-3 \pm 14.7	-1 \pm 14.0	8** \pm 16.9 (\uparrow 133)
Overall (1-21)	37 \pm 21.2	36 \pm 17.6	39 \pm 15.6	54** \pm 20.8 (\uparrow 46)

a Data were extracted from the study report, Tables 7 and 9, pages 95 and 97. Percent difference from controls is presented parenthetically; n=23-25.

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

When compared to concurrent controls, absolute (g/animal/day) food consumption was reduced ($p \leq 0.05$) in the 250 mg/kg dams during GDs 6-15 (\downarrow 9-23%), 6-20 (\downarrow 9%), and 0-20 (\downarrow 9%, Table 3). Absolute food consumption was also decreased during LDs 1-4 (\downarrow 17%, $p \leq 0.01$) at this dose. Relative (g/kg/day) food consumption was reduced ($p \leq 0.01$) in the 250 mg/kg dams during the GD intervals 6-9 (\downarrow 19%), 9-12 (\downarrow 14%), and 15-20 (\downarrow 11%). In addition, relative food consumption was increased ($p \leq 0.05$) at 250 mg/kg during LDs 10-16 (\uparrow 9%) and 10-21 (\uparrow 6%).

Table 3. Selected mean (\pm S.D.) absolute food consumption (g/animal/day) for P females administered isoxaflutole from GD 6 to LD 10.^a

Treatment interval (days)	Dose (mg/kg/day)			
	0	5	25	250
Gestation				
0-3	20 \pm 2.9	20 \pm 1.3	19 \pm 1.7	19 \pm 2.2
6-9	22 \pm 1.7	23 \pm 1.9	22 \pm 2.6	17** \pm 3.2 (\downarrow 23)
12-15	23 \pm 3.6	23 \pm 2.2	23 \pm 2.2	21** \pm 2.9 (\downarrow 9)
Treatment (6-20)	23 \pm 3.1	24 \pm 1.6	24 \pm 2.2	21** \pm 2.1 (\downarrow 9)
Overall (0-20)	23 \pm 2.6	23 \pm 1.3	23 \pm 1.9	21** \pm 1.7 (\downarrow 9)
Lactation				
1-4	36 \pm 5.8	34 \pm 6.7	34 \pm 4.1	30** \pm 7.0 (\downarrow 17)
7-10	51 \pm 5.4	52 \pm 6.6	50 \pm 4.9	48 \pm 6.0
Treatment (1-10)	43 \pm 4.5	43 \pm 5.8	42 \pm 2.9	40 \pm 4.2
Overall (1-21)	55 \pm 3.7	56 \pm 5.3	55 \pm 3.8	54 \pm 3.6

a Data were extracted from the study report Tables 10 and 12, pages 98 and 100. Percent difference from controls is listed parenthetically; n=23-25.

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

3. **Reproductive performance:** Pregnancy rate, number of implantations/dam, gestation length, and sex ratio were comparable between treated and control animals (Table 4).

Table 4. Delivery observations in P females administered isoxaflutole from GD 6 to LD 10. ^a

Observation	Dose (mg/kg/day)			
	0	5	25	250
# Animals Mated	25	25	25	25
# Animals Pregnant	23	25	24	24
Pregnancy Rate (%)	(92)	(100)	(96)	(96)
# Nonpregnant	2	0	1	1
Mean (\pm SD) gestation length (days)	21.6 \pm 0.58	21.5 \pm 0.51	21.4 \pm 0.49	21.9 \pm 0.34
Total # Implantations ^b	368	400	355	396
Mean (\pm SD) Implantations/Dam	16.0 \pm 2.51	16.0 \pm 1.86	15.4 \pm 2.55 ^c	16.5 \pm 2.41
Total # of Litters Examined	23	25	24	24
Sex Ratio (% Male, \pm SD)	50.5 \pm 13.07	48.6 \pm 11.50	47.7 \pm 13.22	51.1 \pm 12.92

- a Data extracted from the study report Table 1, page 73; Table 14, page 102; Table 17, page 105; Table 18, page 106.
b Calculated by reviewers from data presented in this table.
c n=23

4. Maternal postmortem results

- a. **Macroscopic examination:** No treatment-related pathological abnormalities were observed in any treated group.
- b. **Microscopic examination:** Microscopic examinations were not conducted on adult animals.

B. OFFSPRING

1. **Viability and clinical signs:** No treatment-related differences in live litter size, post-natal survival, or sex ratios were observed in any treated group (Table 5). Decreased ($p \leq 0.05$) survival was observed in the 250 mg/kg pups during PNDs 0-1 only (93.2% treated vs. 98.3% controls). No treatment-related clinical signs were observed.

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Table 5. F₁ live litter size and viability. ^a

Observation	Dose (mg/kg/day)			
	0	5	25	250
Number of litters	23	25	24	24
Live litter size (PND 0)	15.2±1.97	15.1±2.22	14.6±2.83	15.6±2.60
Survival (%)				
PND 0	99.0±3.00	98.5±3.32	98.2±4.60	98.9±2.52
PND 0-1	98.3±4.52	97.8±5.38	100±0.0	93.2*±10.16
PND 1-4 ^b	98.9±3.07	99.7±1.33	99.5±1.62	98.2±3.23
PND 4 ^c -7	100±0.0	100±0.0	100±0.0	99.5±2.55
PND 7-14	99.4±2.98	98.4±4.55	100±0.0	99.4±2.92
PND 14-21	100±0.0	99.4±2.86	100±0.0	100±0.0
Sex ratio (% male)	50.5±13.07	48.6±11.50	47.7±13.22	51.1±12.92

a Data extracted from the study report Tables 18 and 19, pages 106 through 108.

b Before culling

c After culling

* Significantly different from controls at p≤0.05

2. **Body weight:** Selected body weights and body weight gains for F₁ pups are presented in Tables 6a and 6b. Body weights were decreased (p≤0.05) in the 250 mg/kg males from PND 1 to 28 (↓7-12%) and from PND 49 to 63 (↓4-5%). In the 250 mg/kg females, body weights were decreased (p≤0.05) sporadically between PND 1 and 35 (↓5-12%). Decreased (p≤0.05) body weight gains were observed sporadically between PNDs 1 and 28 in the 250 mg/kg males (↓6-21%) and only during PNDs 4-7 in the 250 mg/kg females (↓15%). Body weight gains were sporadically increased (p≤0.05) in the 25 and 250 mg/kg females during PNDs 49-70 (↑17-29%); however, these increases were considered to be unrelated to treatment since mean post-weaning body weight gain in these groups was similar to controls.

Table 6a. Mean (± SD) F₁ pup body weights (g). ^a

Post-natal Day	Dose (mg/kg/day)			
	0	5	25	250
Males				
1	7.0±0.78	7.1±0.68	7.0±0.84	6.4*±0.67 (↓9)
7	15.4±2.25	15.4±2.00	15.4±1.93	13.5**±1.70 (↓12)
17	40.1±3.99	41.3±3.77	39.7±4.38	37.1*±3.77 (↓7)
28	88.6±13.02	90.8±9.82	86.3±9.17	82.4**±10.95 (↓7)
49	272.2±30.42	281.7±26.72	276.9±21.71	259.0*±29.83 (↓5)
63	380.1±38.37	391.5±35.5	386.8±28.96	363.2*±37.06 (↓4)
72	426.2±46.36	439.6±39.75	435.3±36.16	408.9±43.08
Females				
1	6.6±0.79	6.6±0.67	6.5±0.75	6.0*±0.65 (↓9)
7	14.5±2.25	14.6±2.09	14.5±1.87	12.8**±1.53 (↓12)
28	82.0±9.98	83.6±8.70	79.9±8.76	77.6**±8.69 (↓5)
35	130.1±13.38	129.7±11.67	128.0±11.29	122.4**±12.55 (↓6)
49	193.3±18.92	194.3±15.20	193.8±17.39	188.2±17.21
72	258.8±28.75	259.9±25.60	265.2±27.20	257.2±24.35

a Data obtained from the study report Table 23, pages 157 through 162. Percent difference from controls is presented

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parenthetically; n = 23-89.

* Significantly different from controls at $p \leq 0.05$ ** Significantly different from controls at $p \leq 0.01$ Table 6b. Selected F₁ pup mean (\pm SD) body weight gains (g).^a

Post-natal Day	Dose (mg/kg/day)			
	0	5	25	250
Males				
1-4	2.8 \pm 0.75	2.9 \pm 0.70	2.8 \pm 0.80	2.2* \pm 0.58 (\downarrow 21)
21-28	35.8 \pm 7.11	36.3 \pm 5.85	35.4 \pm 6.51	33.5* \pm 5.19 (\downarrow 16)
70-72	9.0 \pm 7.59	10.5 \pm 4.73	10.3 \pm 5.29	8.8 \pm 3.94
(Post-weaning) 21-72	374.2 \pm 42.02	384.9 \pm 38.32	383.8 \pm 33.46	359.9 \pm 39.56
Females				
1-4	2.6 \pm 0.82	2.7 \pm 0.70	2.7 \pm 0.76	2.2 \pm 0.53
4-7	5.3 \pm 1.17	5.3 \pm 1.21	5.3 \pm 0.74	4.5* \pm 0.85 (\downarrow 15)
17-21	12.2 \pm 2.64	11.9 \pm 2.53	10.5 \pm 1.76	11.3 \pm 2.12
63-70	14.6 \pm 6.62	15.3 \pm 7.30	18.4* \pm 7.44 (\uparrow 26)	18.8** \pm 6.16 (\uparrow 29)
70-72	4.0 \pm 7.47	4.4 \pm 7.04	3.7 \pm 7.19	3.1 \pm 6.82
(Post-weaning) 21-72	206.8 \pm 26.85	208.7 \pm 24.14	216.5 \pm 26.42	209.4 \pm 23.01

a Data obtained from the study report Table 24, pages 163 through 168. Percent difference from controls is presented parenthetically; n = 23-89.

* Significantly different from controls at $p \leq 0.05$ ** Significantly different from controls at $p \leq 0.01$ **3. Developmental landmarks**

- a. **Sexual maturation:** A slight delay in mean balanopreputial separation (44.1 days treated vs. 42.6 days controls [\uparrow 4%], $p \leq 0.05$) was observed in the 250 mg/kg males (Table 7). No differences in vaginal patency were observed between treated and control F₁ females.

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Table 7. Balanopreputial separation or vaginal patency (mean days \pm S.D.) in F₁ generation males or females.

		Dose (mg/kg/day)				
		0	5	25	250	Historical Controls; n=16
Males						
		42.6 \pm 1.75	41.9 \pm 1.54	42.3 \pm 1.59	44.1* \pm 2.88 (†4)	44.5 \pm 2.28
Females						
		33.2 \pm 1.13	33.6 \pm 1.08	33.2 \pm 0.75	33.2 \pm 1.49	33.2 \pm 1.64

a Data extracted from the study report Tables 25 and 27, pages 169 and 172. Percent difference from controls is presented parenthetically; n=23-25 unless noted otherwise.

* Significantly different from controls at $p \leq 0.05$.

4. Behavioral assessments

a. **Functional observational battery:** No treatment-related FOB effects were observed in any group.

b. **Motor activity:** Mean total and ambulatory motor activities were comparable between treated animals and controls at all time points (Table 8). Habituation was unaffected by treatment. It should be noted that motor activity values were highly variable.

Table 8. Mean (\pm S.D.) total and ambulatory motor activity (counts) in F₁ pups.^a

Post-natal Day		Dose (mg/kg/day)			
		0	5	25	250
Males					
13	Total	336 \pm 169.1	443 \pm 182.3	418 \pm 235.7	387 \pm 166.7
	Ambulatory	49 \pm 51.6	79 \pm 81.6	105 \pm 84.7	64 \pm 78.3
17	Total	425 \pm 282.7	324 \pm 304.9	489 \pm 322.1	860 \pm 702.2
	Ambulatory	146 \pm 153.7	98 \pm 140.6	162 \pm 160.9	395 \pm 451.0
21	Total	682 \pm 307.6	568 \pm 327.5	467 \pm 176.5	549 \pm 280.1
	Ambulatory	239 \pm 128.8	181 \pm 145.1	128 \pm 68.5	184 \pm 127.2
61	Total	1534 \pm 606.9	2070 \pm 429.6	1931 \pm 534.0	1909 \pm 614.4
	Ambulatory	411 \pm 221.9	604 \pm 158.5	587 \pm 256.5	607 \pm 255.2
Females					
13	Total	247 \pm 82.7	368 \pm 333.1	461 \pm 475.2	449 \pm 312.0
	Ambulatory	46 \pm 57.5	77 \pm 174.0	136 \pm 311.6	84 \pm 150.4
17	Total	190 \pm 108.1	294 \pm 189.1	509 \pm 656.0	876 \pm 875.7
	Ambulatory	38 \pm 34.1	88 \pm 77.9	184 \pm 341.5	376 \pm 453.7
21	Total	408 \pm 136.8	618 \pm 389.9	627 \pm 396.9	586 \pm 317.7
	Ambulatory	131 \pm 52.2	169 \pm 132.3	196 \pm 163.4	204 \pm 124.7
61	Total	1403 \pm 451.0	1781 \pm 378.3	1966 \pm 481.4	1695 \pm 478.9
	Ambulatory	509 \pm 200.6	646 \pm 174.6	755 \pm 233.2	657 \pm 189.6

a Data obtained from the study report Table 30, pages 177 and 178.

c. **Auditory startle reflex habituation:** No treatment-related differences in startle response were observed in any treated group compared to controls (Table 9). The decrease observed in T_{max} in

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the 5 mg/kg females at PND 60 (\downarrow 15%, $p \leq 0.05$) was not dose-dependent and considered unrelated to treatment.

Table 9. Mean (\pm SD) auditory startle reflex data. ^a

Observation		Dose (mg/kg/day)			
		0	5	25	250
Males					
PND 20	V_{max} (mv)	161.7 \pm 68.01	193.7 \pm 37.64	168.5 \pm 59.05	161.7 \pm 58.23
	T_{max} (ms)	26.5 \pm 4.67	24.3 \pm 2.12	25.2 \pm 2.35	26.1 \pm 3.26
	V_{ave} (mv)	35.4 \pm 12.45	44.4 \pm 7.29	37.7 \pm 11.89	37.3 \pm 13.90
PND 60	V_{max} (mv)	195.5 \pm 90.87	169.5 \pm 82.87	207.4 \pm 172.51	154.2 \pm 87.72
	T_{max} (ms)	30.3 \pm 4.38	34.4 \pm 5.27	30.7 \pm 4.87	32.4 \pm 3.43
	V_{ave} (mv)	42.7 \pm 18.74	36.7 \pm 15.93	42.3 \pm 31.41	32.8 \pm 17.09
Females					
PND 20	V_{max} (mv)	198.3 \pm 37.67	173.7 \pm 57.69	204.1 \pm 95.33	171.1 \pm 54.79
	T_{max} (ms)	23.2 \pm 2.00	23.2 \pm 2.35	22.7 \pm 2.93	23.3 \pm 3.02
	V_{ave} (mv)	44.4 \pm 8.15	38.9 \pm 10.18	44.1 \pm 20.36	38.0 \pm 11.85
PND 60	V_{max} (mv)	106.8 \pm 56.02	171.7 \pm 119.51	86.2 \pm 41.06	112.4 \pm 59.53
	T_{max} (ms)	33.6 \pm 4.46	28.6 \pm 2.98* (\downarrow 15)	34.5 \pm 5.15	32.7 \pm 4.59
	V_{ave} (mv)	21.5 \pm 10.95	35.1 \pm 23.66	18.6 \pm 9.25	22.4 \pm 11.43

a Data obtained from Study Report Table 29, pages 175-176; n=10. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).

* Statistically different from control, $p \leq 0.05$

- d. **Learning and memory testing:** No treatment-related differences in swimming ability, learning, or memory were noted in any treated group relative to concurrent controls (Tables 10a and 10b). Mean Day 1 swimming ability (PND 62) was decreased (increased time) relative to concurrent controls in the 250 mg/kg males (7.35 sec. treated vs. 5.76 sec. controls, $p \leq 0.05$). However, in the absence of effects during the actual test (swimming maze), this finding was considered to be incidental.

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Table 10a. Mean (\pm SD) times in Biel swimming trials - males.^a

Test Day/Parameter		Dose (mg/kg/day)			
		0	5	25	250
PND 22					
Day 1	Swimming ability (sec)	17.12 \pm 14.499	14.02 \pm 3.980	12.02 \pm 5.014	12.88 \pm 3.857
Day 2	Time (sec)	96.63 \pm 55.250	100.10 \pm 60.746	90.30 \pm 47.861	102.50 \pm 44.181
Path A Trial 1	Errors	16 \pm 8.0	16 \pm 11.2	15 \pm 11.2	17 \pm 8.0
Day 2	Time (sec)	101.38 \pm 75.863	59.90 \pm 28.683	58.40 \pm 28.701	67.85 \pm 41.340
Path A Trial 2	Errors	14 \pm 11.2	10 \pm 5.9	10 \pm 5.2	11 \pm 9.2
Day 3	Time (sec)	86.78 \pm 61.852	44.37 \pm 20.663	62.35 \pm 33.568	65.51 \pm 60.052
Path A Trial 3	Errors	17 \pm 12.2	9 \pm 6.6	13 \pm 8.3	13 \pm 14.1
Day 3	Time (sec)	56.39 \pm 46.604	53.65 \pm 56.256	52.58 \pm 30.048	59.79 \pm 49.190
Path A Trial 4	Errors	11 \pm 6.2	11 \pm 13.3	12 \pm 8.8	14 \pm 13.2
Day 4	Time (sec)	137.83 \pm 68.688	158.61 \pm 42.431	147.62 \pm 58.353	167.27 \pm 26.953
Path B Trial 5	Errors	22 \pm 15.3	24 \pm 7.6	21 \pm 10.9	32 \pm 9.0
Day 4	Time (sec)	124.54 \pm 72.838	78.99 \pm 55.317	136.19 \pm 57.468	116.84 \pm 56.607
Path B Trial 6	Errors	22 \pm 18.3	13 \pm 10.0	23 \pm 10.3	23 \pm 12.7
Day 5	Time (sec)	110.89 \pm 62.564	114.74 \pm 70.561	111.44 \pm 65.167	107.57 \pm 57.917
Path B Trial 7	Errors	17 \pm 11.2	20 \pm 10.8	20 \pm 11.7	18 \pm 12.0
Day 5	Time (sec)	56.58 \pm 52.106	119.57 \pm 69.004	77.97 \pm 47.557	79.72 \pm 71.519
Path B Trial 8	Errors	11 \pm 10.4	24 \pm 14.9	16 \pm 7.3	19 \pm 19.3
Day 6	Time (sec)	91.99 \pm 76.383	107.89 \pm 65.254	104.46 \pm 71.406	86.66 \pm 66.697
Path B Trial 9	Errors	14 \pm 15.2	18 \pm 9.7	21 \pm 12.7	14 \pm 10.2
Day 6	Time (sec)	72.00 \pm 70.124	72.36 \pm 53.344	67.56 \pm 67.968	72.06 \pm 53.752
Path B Trial 10	Errors	13 \pm 13.3	17 \pm 14.3	12 \pm 14.0	17 \pm 12.9
Day 7	Time (sec)	72.90 \pm 46.157	62.73 \pm 47.978	65.09 \pm 51.095	54.85 \pm 32.922
Path A (probe) Trial 11	Errors	22 \pm 14.0	17 \pm 13.5	18 \pm 16.7	15 \pm 7.3
Day 7	Time (sec)	52.35 \pm 21.434	58.14 \pm 43.686	41.59 \pm 26.620	36.78 \pm 21.129
Path A (probe) Trial 12	Errors	12 \pm 5.9	14 \pm 13.9	9 \pm 10.2	8 \pm 7.3
Overall Biel (Trials 1-10)	Time (sec)	93.50 \pm 23.780	91.02 \pm 19.695	90.58 \pm 25.706	92.58 \pm 22.147
	Errors	16 \pm 3.8	16 \pm 4.1	16 \pm 4.6	18 \pm 5.1
Overall probe (Trials 11-12)	Time (sec)	62.63 \pm 22.436	60.44 \pm 34.395	53.34 \pm 37.488	45.82 \pm 17.791
	Errors	17 \pm 7.3	16 \pm 10.9	14 \pm 12.7	12 \pm 4.7

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Test Day/Parameter		Dose (mg/kg/day)			
		0	5	25	250
PND 62 ^a					
Day 1	Swimming ability (sec)	5.76±1.271	6.13±1.251	5.34±0.947	7.35±1.679* (128)
Day 2	Time (sec)	109.81±36.242	95.72±45.942	79.88±48.036	94.58±55.871
Path A Trial 1	Errors	21±7.3	17±11.8	13±8.4	16±8.9
Day 2	Time (sec)	54.15±33.351	51.98±40.280	44.59±28.018	57.84±49.867
Path A Trial 2	Errors	12±8.8	11±7.5	9±6.4	12±10.0
Day 3	Time (sec)	47.32±29.021	30.54±12.127	34.83±35.638	53.17±52.773
Path A Trial 3	Errors	9±7.5	5±2.2	6±5.8	9±10.9
Day 3	Time (sec)	20.36±7.136	23.84±16.064	23.93±24.782	24.93±18.154
Path A Trial 4	Errors	6±3.7	5±4.0	4±6.7	5±6.3
Day 4	Time (sec)	130.87±57.626	152.53±45.189	164.50±41.367	138.45±58.303
Path B Trial 5	Errors	19±9.6	26±11.0	31±10.7	23±11.7
Day 4	Time (sec)	71.09±55.932	109.08±71.494	99.88±66.538	120.90±73.104
Path B Trial 6	Errors	13±10.5	22±16.1	19±14.8	22±14.0
Day 5	Time (sec)	60.22±54.105	93.38±65.872	72.87±49.980	120.40±66.146
Path B Trial 7	Errors	11±11.1	16±11.6	11±10.2	20±15.0
Day 5	Time (sec)	73.33±63.689	44.09±30.979	32.23±31.400	33.47±22.320
Path B Trial 8	Errors	13±11.2	8±7.0	5±6.6	7±7.4
Day 6	Time (sec)	44.86±53.783	21.87±14.776	62.29±53.209	31.65±21.028
Path B Trial 9	Errors	6±9.5	3±3.2	8±8.2	5±5.3
Day 6	Time (sec)	26.47±27.818	15.52±8.072	41.56±35.965	21.14±11.500
Path B Trial 10	Errors	4±7.3	2±2.3	7±8.5	3±2.9
Day 7	Time (sec)	45.94±36.922	71.50±56.031	63.34±48.703	61.05±33.974
Path A (probe) Trial 11	Errors	9±9.1	15±11.3	15±14.3	15±11.3
Day 7	Time (sec)	31.60±18.636	40.01±45.352	57.11±65.432	37.02±17.072
Path A (probe) Trial 12	Errors	7±5.8	6±9.7	11±13.3	9±5.0
Overall Biel (Trials 1-10)	Time (sec)	63.85±22.055	63.87±10.624	66.39±21.309	67.85±20.814
	Errors	11±3.8	12±2.4	11±3.9	13±3.2
Overall probe (Trials 11-12)	Time (sec)	38.77±24.366	55.76±44.162	60.23±47.272	49.04±22.204
	Errors	8±6.7	11±8.5	13±11.7	12±7.2

a Data obtained from Study Report Tables 31, pages 179-182 and 32, pages 187-190; n=10. Path A = forward through maze; Path B = reverse through maze; Time = mean time to escape; Error = all four feet into an incorrect channel.

* Statistically different from control at p≤0.05

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Table 10b. Mean (\pm SD) times in Biel swimming trials - females. ^a

Test Day/Parameter		Dose (mg/kg/day)			
		0	5	25	250
PND 22					
Day 1	Swimming ability (sec)	11.95 \pm 3.504	16.15 \pm 9.387	15.69 \pm 6.742	12.35 \pm 2.920
Day 2	Time (sec)	108.17 \pm 74.589	91.27 \pm 47.691	117.10 \pm 44.717	106.37 \pm 63.789
Path A Trial 1	Errors	17 \pm 11.9	16 \pm 9.6	20 \pm 9.5	19 \pm 13.0
Day 2	Time (sec)	70.37 \pm 40.593	82.83 \pm 55.094	70.83 \pm 39.965	94.57 \pm 39.194
Path A Trial 2	Errors	13 \pm 8.8	14 \pm 9.4	11 \pm 6.6	19 \pm 9.3
Day 3	Time (sec)	54.71 \pm 42.066	43.45 \pm 38.550	67.51 \pm 39.301	70.79 \pm 50.600
Path A Trial 3	Errors	9 \pm 7.2	8 \pm 10.5	14 \pm 9.4	15 \pm 14.6
Day 3	Time (sec)	46.46 \pm 37.747	41.31 \pm 49.793	58.06 \pm 40.184	51.10 \pm 34.217
Path A Trial 4	Errors	9 \pm 8.7	7 \pm 9.9	12 \pm 11.2	9 \pm 8.6
Day 4	Time (sec)	147.97 \pm 42.135	118.86 \pm 60.061	146.80 \pm 44.401	150.03 \pm 57.699
Path B Trial 5	Errors	25 \pm 8.6	20 \pm 10.2	22 \pm 6.8	23 \pm 10.3
Day 4	Time (sec)	104.66 \pm 58.763	91.59 \pm 56.770	136.59 \pm 52.936	90.09 \pm 66.731
Path B Trial 6	Errors	19 \pm 11.1	19 \pm 13.0	23 \pm 12.9	20 \pm 17.5
Day 5	Time (sec)	91.88 \pm 51.210	94.22 \pm 56.340	116.94 \pm 46.361	113.47 \pm 65.480
Path B Trial 7	Errors	17 \pm 10.2	20 \pm 12.0	25 \pm 10.5	20 \pm 11.1
Day 5	Time (sec)	53.78 \pm 49.342	83.65 \pm 60.256	93.13 \pm 54.905	63.60 \pm 64.282
Path B Trial 8	Errors	10 \pm 8.6	17 \pm 11.0	17 \pm 10.8	14 \pm 14.6
Day 6	Time (sec)	59.81 \pm 49.567	82.31 \pm 66.331	80.63 \pm 57.920	74.27 \pm 56.595
Path B Trial 9	Errors	10 \pm 9.0	15 \pm 10.8	14 \pm 10.7	13 \pm 11.5
Day 6	Time (sec)	39.97 \pm 40.670	59.97 \pm 52.754	58.10 \pm 52.687	102.44 \pm 58.907
Path B Trial 10	Errors	6 \pm 6.9	12 \pm 10.4	10 \pm 8.6	22 \pm 13.4
Day 7	Time (sec)	78.51 \pm 58.118	54.39 \pm 32.615	84.72 \pm 53.276	77.74 \pm 46.385
Path A (probe) Trial 11	Errors	20 \pm 11.9	15 \pm 10.0	24 \pm 18.6	23 \pm 15.4
Day 7	Time (sec)	64.78 \pm 61.931	44.81 \pm 22.516	71.60 \pm 39.537	34.45 \pm 28.660
Path A (probe) Trial 12	Errors	17 \pm 17.7	12 \pm 6.8	16 \pm 9.7	7 \pm 8.6
Overall Biel (Trials 1-10)	Time (sec)	77.78 \pm 17.284	78.95 \pm 18.893	94.57 \pm 18.353	91.47 \pm 23.035
	Errors	13 \pm 3.5	15 \pm 3.3	17 \pm 3.2	18 \pm 4.6
Overall probe (Trials 11-12)	Time (sec)	74.11 \pm 47.589	49.60 \pm 10.968	78.16 \pm 40.639	56.10 \pm 35.461
	Errors	19 \pm 8.2	14 \pm 4.5	21 \pm 11.7	15 \pm 11.5

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Test Day/Parameter		Dose (mg/kg/day)			
		0	5	25	250
PND 62					
Day 1	Swimming ability (sec)	8.16±3.360	8.53±1.971	8.65±3.451	7.60±1.779
Day 2	Time (sec)	77.95±60.604	68.62±26.602	66.31±56.166	76.11±50.187
Path A Trial 1	Errors	15±10.9	12±6.1	11±12.6	15±8.5
Day 2	Time (sec)	65.33±58.069	65.83±52.220	57.38±52.689	55.00±42.780
Path A Trial 2	Errors	13±13.6	13±11.1	10±9.4	11±9.2
Day 3	Time (sec)	45.75±33.949	46.25±41.737	37.44±35.504	53.19±34.298
Path A Trial 3	Errors	8±4.2	8±7.4	5±3.4	10±7.5
Day 3	Time (sec)	31.36±22.881	43.68±45.682	28.14±28.722	47.89±41.921
Path A Trial 4	Errors	6±5.7	9±14.1	4±6.1	12±12.6
Day 4	Time (sec)	139.53±67.970	112.73±49.766	133.59±47.187	149.50±56.578
Path B Trial 5	Errors	24±13.8	19±7.6	25±6.7	27±11.6
Day 4	Time (sec)	92.44±61.246	71.04±57.392	91.88±68.531	122.37±62.803
Path B Trial 6	Errors	16±12.8	13±11.6	16±13.8	24±14.1
Day 5	Time (sec)	60.64±46.830	71.07±64.558	75.81±65.797	104.53±73.381
Path B Trial 7	Errors	9±9.2	10±12.4	12±12.5	22±16.8
Day 5	Time (sec)	26.69±16.406	40.71±32.215	45.32±53.622	50.34±54.202
Path B Trial 8	Errors	3±3.4	6±6.3	7±10.3	8±11.2
Day 6	Time (sec)	27.39±13.418	45.42±31.865	38.47±29.906	63.66±66.376
Path B Trial 9	Errors	3±4.2	7±7.0	5±4.8	12±15.3
Day 6	Time (sec)	32.39±16.742	17.34±7.528	31.56±25.776	52.89±47.545
Path B Trial 10	Errors	6±4.2	1±1.6	4±6.1	10±9.9
Day 7	Time (sec)	43.86±44.061	52.50±49.604	42.23±33.559	32.29±17.276
Path A (probe) Trial 11	Errors	9±9.7	9±10.6	7±4.5	6±4.4
Day 7	Time (sec)	46.24±39.342	57.80±50.463	53.24±50.268	33.37±14.727
Path A (probe) Trial 12	Errors	10±12.2	10±12.0	10±14.0	8±8.6
Overall Biel (Trials 1-10)	Time (sec)	59.95±19.748	58.27±20.134	61.79±29.904	77.55±27.998
	Errors	10±3.9	10±3.7	10±4.6	15±5.3
Overall probe (Trials 11-12)	Time (sec)	45.05±36.357	55.15±39.753	47.74±26.359	32.83±9.324
	Errors	10±9.3	10±8.1	8±6.1	7±4.4

- a Data obtained from Study Report Tables 31, pages 183-186 and 32, pages 191-194; n=10. Path A = forward through maze; Path B = reverse through maze; Time = mean time to escape; Error = all four feet into an incorrect channel.
 * Statistically different from control at p≤0.05

5. Postmortem results

- a. **Brain weights:** No treatment-related differences in brain weights or dimensions were noted. Absolute brain weight was decreased (p≤0.05) in the 250 mg/kg males and females on PND 11 (↓11-12%). Relative (to body) brain weight was increased (p≤0.05) in the 250 mg/kg males on PND 72 (↑10%) while absolute brain weight was unaffected; this increase was considered to be due to decreased (p≤0.05) terminal body weights in these animals (↓14%). Decreased brain width was noted in the 5 mg/kg males on PND 11 (↓4%, p≤0.05); however, this decrease was not dose-dependent and therefore unrelated to treatment.

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Table I1. Mean (\pm SD) brain weights and dimensions in F₁ rats. ^a

Parameter	Dose (mg/kg/day)			
	0	5	25	250
Males				
PND 11				
Terminal Body Weight (g)	25 \pm 3.3	24 \pm 2.9	24 \pm 4.7	22 \pm 2.8
Absolute Brain weight (g)	1.18 \pm 0.065	1.16 \pm 0.108	1.10 \pm 0.121	1.05 \pm 0.066*(↓11)
Relative (to body) Brain (g/100 g)	4.697 \pm 0.5114	4.787 \pm 0.4028	4.647 \pm 0.5770	4.888 \pm 0.5136
Brain Length (mm)	15.4 \pm 0.47	15.0 \pm 0.47	14.8 \pm 0.76	15.2 \pm 0.41
Brain Width (mm)	12.7 \pm 0.40	12.2 \pm 0.43*(↓4)	12.6 \pm 0.57	12.2 \pm 0.40
PND 72				
Terminal Body Weight (g)	451 \pm 28.9	440 \pm 34.4	447 \pm 39.1	388 \pm 30.3*(↓14)
Absolute Brain weight (g)	1.95 \pm 0.073	1.99 \pm 0.109	1.94 \pm 0.090	1.85 \pm 0.111
Relative (to body) Brain (g/100 g)	0.435 \pm 0.0308	0.454 \pm 0.0313	0.438 \pm 0.0472	0.479 \pm 0.0272*(↑10)
Brain Length (mm)	20.3 \pm 0.30	20.7 \pm 0.54	20.3 \pm 0.55	20.4 \pm 0.50
Brain Width (mm)	14.7 \pm 0.37	15.1 \pm 0.40	15.1 \pm 0.38	14.6 \pm 0.46
Females				
PND 11				
Terminal Body Weight (g)	23 \pm 3.2	23 \pm 3.2	22 \pm 3.0	19 \pm 5.0
Absolute Brain weight (g)	1.08 \pm 0.102	1.09 \pm 0.114	1.08 \pm 0.052	0.95 \pm 0.126*(↓12)
Relative (to body) Brain (g/100 g)	4.834 \pm 0.4477	4.672 \pm 0.3600	4.918 \pm 0.5566	5.248 \pm 1.0823
Brain Length (mm)	14.8 \pm 0.78	14.5 \pm 0.48	14.7 \pm 0.45	14.8 \pm 0.78
Brain Width (mm)	12.2 \pm 0.37	12.1 \pm 0.62	12.3 \pm 0.60	11.9 \pm 0.66
PND 72				
Terminal Body Weight (g)	253 \pm 24.4	254 \pm 14.4	247 \pm 29.7	255 \pm 16.5
Absolute Brain weight (g)	1.82 \pm 0.089	1.81 \pm 0.082	1.78 \pm 0.064	1.75 \pm 0.069
Relative (to body) Brain (g/100 g)	0.723 \pm 0.0661	0.715 \pm 0.0531	0.729 \pm 0.0837	0.687 \pm 0.0569
Brain Length (mm)	19.9 \pm 0.30	20.0 \pm 0.43	19.8 \pm 0.70	19.8 \pm 0.75
Brain Width (mm)	14.6 \pm 0.42	14.5 \pm 0.47	14.2 \pm 0.30	14.3 \pm 0.42

- a Data obtained from Study Report Tables 38-41, pages 200-207; n=10, except for the control males on PND 11 where n=9. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).
 * Statistically different from control at p \leq 0.05

b) Neuropathology

- 1) **Macroscopic examination:** No treatment-related gross pathological findings were noted in any treated group.
- 2) **Microscopic examination:** No treatment-related microscopic findings were noted. Morphometric evaluations were not performed.

Note: The report stated that the evaluation of brains by light microscopy did not reveal any structural abnormalities, nor were there any clear functional differences between the control or treated groups. Therefore, morphometric analyses on PND 11 and 72 were not performed as the sponsor requested.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: It was concluded that maternal toxicity at 250 mg/kg/day was characterized by decreased body weight gain and food consumption. Developmental and/or neonatal toxicity at 250 mg/kg/day was characterized by decreased postnatal survival for PND 0-1 and decreased pup body weights. The maternal NOAEL was 25 mg/kg/day. The reproductive NOAEL was 250 mg/kg/day. The developmental neurotoxicity NOAEL was 250 mg/kg/day.

B. REVIEWER'S COMMENTS: No unscheduled parental deaths occurred during the study. Clinical signs, gross pathology, pregnancy rate, number of implantations/dam, gestation length, and sex ratio were unaffected by treatment. Reproductive function was not evaluated.

In the 250 mg/kg dams, body weights were slightly decreased ($p \leq 0.05$) during GDs 9-15 ($\downarrow 4-6\%$) and LDs 1-10 ($\downarrow 7-10\%$). Body weight gains were decreased ($p \leq 0.01$) during GDs 6-9 ($\downarrow 91\%$) and 9-12 ($\downarrow 39\%$). Body weight gains were also decreased for the entire gestation treatment interval (GDs 6-20; $\downarrow 14\%$, $p \leq 0.01$) and overall gestation (GDs 0-20; $\downarrow 11\%$, $p \leq 0.05$). During the lactation treatment interval (LDs 1-10), body weight gains were comparable to controls in all treated groups. Body weight gains were increased ($p \leq 0.01$) during LDs 10-21 ($\uparrow 133\%$) and LDs 1-21 ($\uparrow 46\%$). Absolute (g/animal/day) food consumption was reduced ($p \leq 0.05$) during GDs 6-15 ($\downarrow 9-23\%$), 6-20 ($\downarrow 9\%$), and 0-20 ($\downarrow 9\%$). Absolute food consumption was also decreased during LDs 1-4 ($\downarrow 17\%$, $p \leq 0.01$). Relative (g/kg/day) food consumption was reduced ($p \leq 0.01$) during the GD intervals 6-9 ($\downarrow 19\%$), 9-12 ($\downarrow 14\%$), and 15-20 ($\downarrow 11\%$). However, relative food consumption was increased ($p \leq 0.05$) at 250 mg/kg after treatment during LDs 10-16 ($\uparrow 9\%$) and 10-21 ($\uparrow 6\%$).

The maternal LOAEL is 250 mg/kg/day based on decreased body weights, body weight gains, and food consumption. The maternal NOAEL is 25 mg/kg/day.

No treatment-related differences in live litter size, post-natal survival, sex ratios, clinical signs, or sexual maturation were observed in any treated group. Pup swimming ability, learning, memory, motor activity, auditory startle response, brain weights and dimensions, and neuropathology were unaffected by the test substance.

Body weights at 5 and 25 mg/kg/day were comparable to that of the controls. At 250 mg/kg, body weights were decreased ($p \leq 0.05$) in the males from PND 1 to 28 ($\downarrow 7-12\%$) and from PND 49 to 63 ($\downarrow 4-5\%$). In the females, body weights were decreased ($p \leq 0.05$) sporadically between PND 1 and 35 ($\downarrow 5-12\%$). Decreased ($p \leq 0.05$) body weight gains were observed sporadically in the males between PNDs 1 and 28 ($\downarrow 6-21\%$) and only during PNDs 4-7 in the females ($\downarrow 15\%$). Absolute brain weights were also reduced (11-12%) in males and females on PND 11 at 250 mg/kg/day, an effect likely related to the decreased body weights and body weight gains observed in these animals.

A NOAEL/LOAEL for offspring can not be determined due to the lack of morphometric analyses in this study.

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C. STUDY DEFICIENCIES: The following deficiencies were noted:

- Positive control data that validate the procedures and observers of the performing lab to assess motor activity, neurotoxicity and behavioral effects were not provided.
- Pupillary size and reaction to light were not evaluated as part of the neurotoxicity screening.
- Morphometric evaluation of the brain was not performed.

DATA EVALUATION RECORD

ISOXAFLUTOLE (IFT)

Study Type ('83-6a): Developmental Neurotoxicity Study in the Rat

Work Assignment No. 4-02-190A (Formerly 3-01-111A) (MRID 45215701)

Prepared for

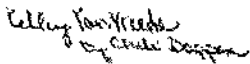
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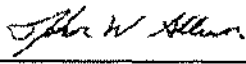
Primary Reviewer:

Kelley Van Vreede, M.S.

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Date: 8/19/02


Secondary Reviewer:

John Allran, M.S.

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Date: 8/19/02

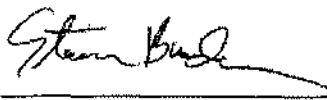
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Steven Brecher, Ph.D.

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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In the 250 mg/kg dams, body weights were slightly decreased ($p \leq 0.05$) during GDs 9-15 (decr 4-6%) and LDs 1-10 (decr 7-10%). Body weight gains were decreased ($p \leq 0.01$) during GDs 6-9 (decr 91%) and 9-12 (decr 39%). Body weight gains were also decreased for the entire gestation treatment interval (GDs 6-20; decr 14%, $p \leq 0.01$) and overall gestation (GDs 0-20; decr 11%, $p \leq 0.05$).

During the lactation treatment interval (LDs 1-10), body weight gains were comparable to controls in all treated groups. Body weight gains were increased ($p \leq 0.01$) during post-treatment (LDs 10-21; inc 133%), resulting in increased body weight gains during the overall lactation interval (LDs 1-21; inc 46%). Absolute (g/animal/day) food consumption was reduced ($p \leq 0.05$) during GDs 6-15 (decr 9-23%), 6-20 (decr 9%), and 0-20 (decr 9%). Absolute food consumption was also decreased during LDs 1-4 (decr 17%, $p \leq 0.01$). Relative (g/kg/day) food consumption was reduced ($p \leq 0.01$) during the GD intervals 6-9 (decr 19%), 9-12 (decr 14%), and 15-20 (decr 11%). In addition, relative food consumption was increased ($p \leq 0.05$) post-treatment during LDs 10-16 (incr 9%) and 10-21 (incr 6%). No treatment-related findings were observed in dams treated with 25 or 5 mg/kg a.i.

The maternal LOAEL is 250 mg/kg/day based on decreased body weights, body weight gains, and food consumption. The maternal NOAEL is 25 mg/kg/day.

No treatment-related differences in live litter size, post-natal survival, sex ratios, clinical signs, or sexual maturation were observed in any treated group. Pup swimming ability, learning, memory, motor activity, auditory startle response, brain weights and dimensions, and neuropathology were unaffected by the test substance.

In the 250 mg/kg pups, body weights were decreased ($p \leq 0.05$) in the males from PND 1 to 28 (decr 7-12%) and from PND 49 to 63 (decr 4-5%). In the females, body weights were decreased ($p \leq 0.05$) sporadically between PND 1 and 35 (decr 5-12%). Decreased ($p \leq 0.05$) body weight gains were observed sporadically in the males between PNDs 1 and 28 (decr 6-21%) and only during PNDs 4-7 in the females (decr 15%). Absolute brain weights were also reduced (11-12%) in males and females on PND 11 at 250 mg/kg/day, an effect likely related to the decreased body weights and body weight gains observed in these animals. Body weights of offspring at the 5 and 25 mg/kg/day groups were comparable to that of the controls.

A NOAEL/LOAEL for offspring cannot be determined due to the lack of morphometric analyses in this study.

This study is classified as **Unacceptable/Guideline due to the lack of morphometric analyses and therefore does not satisfy the guideline requirement** (OPPTS 870.6300; OECD 426) for a developmental neurotoxicity study in rats

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

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Subsequently, ten pups/sex/group were selected for evaluation of neurobehavioral testing, and neuropathological examination. Pups not selected for behavioral evaluations were sacrificed on PND 28 or 29.

3. **Mating procedure:** At approximately 84 days of age, females were paired with males (1:1) for mating. Successful mating was determined by the presence of a copulatory plug or sperm in a vaginal smear. The day on which successful mating was determined was designated as gestation day (GD) 0. Following successful mating, females were housed individually in plastic maternity cages with nesting material. The females and offspring were housed together in these cages through lactation day (LD) 21.
4. **Animal Assignment:** Mated females were randomly assigned (stratified by body weight) to test groups as shown in Table 1. Dams were assigned to functional observation testing as shown.

Offspring were assigned to testing subgroups at the time of litter standardization on PND 4 (Table 1).

Table 1. Study design. ^a

Experimental Parameter	Dose (mg/kg/day)			
	0	5	25	250
Maternal Animals				
No. of maternal animals assigned	25	25	25	25
FOB (GDs 6, 12; LDs 4, 7)	10	10	10	10
Offspring				
Detailed clinical/FOB (PND 4, 11, 21, 35, 45, 60)	10/sex	10/sex	10/sex	10/sex
Motor activity (PND 13, 17, 21, 61±2 days)	10/sex	10/sex	10/sex	10/sex
Auditory startle habituation (PND 20, 60±2 days)	10/sex	10/sex	10/sex	10/sex
Learning and memory (PND 22, 62)	10/sex	10/sex	10/sex	10/sex
Brain weight				
PND 11	10/sex	10/sex	10/sex	10/sex
PND 72 ^b	10/sex	10/sex	10/sex	10/sex
Neuropathology				
PND 11	10/sex	10/sex	10/sex	10/sex
PND 72 ^b	10/sex	10/sex	10/sex	10/sex

a Data obtained from Study Report pages 27, 35, 38, 40, 41, and 42.

b Animals were randomly selected from those pups dedicated to motor activity, auditory startle, and learning and memory tests.

5. **Dose selection rationale:** No dose selection rationale was provided.
6. **Dosage administration:** Doses were administered daily via gavage at 5 mL/kg from GD 6 through LD 10. Dosing was based on the most recent body weight determination.
7. **Dosage preparation and analysis:** Dose formulations were prepared weekly by weighing the test substance and diluting it to the desired concentrations with 1% methylcellulose. The dose formulations were stored at room temperature and stirred continuously during use. Homogeneity and stability were determined prior to the initiation of dosing on representative batches of 1, 5,

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FUNCTIONAL OBSERVATIONS	
Ease of removal from cage	Salivation
Lacrimation/chromodacryorrhea	Fur appearance
Piloerection	Respiratory rate/character
Palpebral closure	Mucous membranes/eye/skin color
Red/crusty deposits	Muscle tone
Eye prominence	Gait
Mobility	Arousal
Convulsions/tremors	Urination/defecation
Grooming	Backing
Bizarre/stereotypic behavior	
Ease of handling	

P females that did not deliver a litter were sacrificed on presumed GD 25, necropsied, and examined for gross lesions and evidence of pregnancy. All other P dams were sacrificed on LD 21 and subjected to a gross pathological examination. Tissues were preserved in 10% neutral-buffered formalin for possible future histopathological examination.

b. Offspring

- 1) Litter observations: Pups were evaluated daily for mortality and morbidity. Clinical observations and body weights were recorded on PNDs 1, 4, 7, 11, 14, 17, and 21, and then weekly thereafter until sacrifice. Post-weaning food consumption was not reported. The following additional litter observations (X) were made (Table 2):

Table 2. Litter observations. ^a

Observation	Post-natal Day							
	0	1	4	7	11	14	17	21
Number of live pups ^b	X	X	X	X	X	X	X	X
Pup weight		X	X	X	X	X	X	X
Clinical observations		X	X	X	X	X	X	X
Number of dead pups ^b	X	X	X	X	X	X	X	X
Sex of each pup	X		X		X			X

a Data obtained from the study report, pages 34-36.

b Observed daily.

On PND 4, litters were standardized by randomly selecting 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were weighed, killed, and discarded. It was stated that litters that did not meet the sex ratio criteria (M/F ratios of 4:4, 5:3, or 3:5) were not to be used for neurobehavioral or neuropathological evaluations and were to be killed and necropsied on PND 4. However, the litter from one 25 mg/kg dam (No. 25927) had a male/female ratio of 2:6 and the pups were not euthanized on PND 4; furthermore, female pup (No. 9) was selected for brain weights on PND 11, females pups (Nos. 2, 5, 6, 7, and 10) were euthanized on PND 28, and males pups (Nos. 1 and 3) were euthanized on PND 72 (not selected for brain weights).

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the number of errors were tabulated for Phases 2 and 3.

2. Postmortem observations

- a. **Maternal animals:** Maternal animals were sacrificed via CO₂ asphyxiation on LD 21 and subjected to gross necropsy. The number of former implantation sites was recorded and tissues were preserved in 10% neutral buffered formalin for possible future histopathological examination.
- b. **Offspring:** The offspring selected for brain weight or neuropathological evaluation were sacrificed on PND 11 or 72. These animals were subjected to postmortem examinations as described below.

On PND 11, 10 pups/sex/group (1 pup/sex/litter) were sacrificed by perfusion fixation and subjected to neuropathological examination. The brains (with olfactory bulbs) were removed, weighed, and measured. Sections from all major brain regions (including olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain, brainstem, and cerebellum) were trimmed, processed into paraffin blocks, sectioned, and stained with hematoxylin and eosin. Tissues from the control and 250 mg/kg pups were examined microscopically.

On PND 72, 10 pups/sex/group (1 pup/sex/litter) were randomly selected from the pups subjected to motor activity, auditory startle response, and learning and memory tests; euthanized by carbon dioxide inhalation; and perfused *in situ*. The central and peripheral nervous system and tissues were dissected, preserved, embedded in paraffin or plastic, sectioned, and stained with hematoxylin and eosin. The following CHECKED (X) tissues from the control and 250 mg/kg pups were examined microscopically:

CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM	
	BRAIN		SCIATIC NERVE
X	Olfactory bulbs	X	Sciatic Nerves
X	Cerebral cortex		OTHER
X	Midbrain		
X	Cerebellum	X	Sural Nerve
X	Hippocampus	X	Tibial Nerve
X	Medulla oblongata	X	Peroneal Nerve
X	Basal ganglion	X	Lumbar dorsal root ganglion
X	Thalamus	X	Lumbar dorsal root fibers
X	Hypothalamus	X	Lumbar ventral root fibers
	SPINAL CORD	X	Cervical dorsal root ganglion
X	Cervical swelling	X	Cervical dorsal root fibers
X	Lumbar swelling	X	Cervical ventral root fibers
	OTHER		
X	Gasserian Ganglion		
X	Trigeminal nerves		
X	Optic nerves		
X	Eyes		
X	Skeletal muscle		

the study. No treatment-related clinical or FOB signs were noted in any group.

2. **Body weight and food consumption:** Body weights and body weight gains for the P females are presented in Tables 2a and 2b. Body weights were slightly decreased ($p \leq 0.05$) in the 250 mg/kg P females during gestation days (GDs) 9-15 ($\downarrow 4-6\%$) and lactation days (LDs) 1-10 ($\downarrow 7-10\%$). Body weight gains were decreased ($p \leq 0.01$) in the 250 mg/kg P females during GDs 6-9 ($\downarrow 91\%$) and 9-12 ($\downarrow 39\%$). Body weight gains were also decreased for the entire gestation treatment interval (GDs 6-20; $\downarrow 14\%$, $p \leq 0.01$) and overall gestation (GDs 0-20; $\downarrow 11\%$, $p \leq 0.05$). During the lactation treatment interval (LDs 1-10), body weight gains were comparable to controls in all treated groups. Body weight gains were increased ($p \leq 0.01$) in the 250 mg/kg P females during post-treatment LDs 10-21 ($\uparrow 133\%$), resulting in increased body weight gains during LDs 1-21 ($\uparrow 46\%$).

Table 2a. Selected mean (\pm S.D.) body weights (g) for P females administered isoxaflutole from GD 6 to LD 10.^a

Treatment interval (days)	Dose (mg/kg/day)			
	0	5	25	250
Gestation				
0	261 \pm 14.7	258 \pm 11.9	256 \pm 13.4	258 \pm 14.1
6	294 \pm 19.8	292 \pm 11.9	286 \pm 15.4	291 \pm 15.1
9	305 \pm 18.7	304 \pm 13.3	297 \pm 17.9	292* \pm 12.2 ($\downarrow 4$)
15	341 \pm 28.4	334 \pm 21.7	332 \pm 21.3	320** \pm 15.4 ($\downarrow 6$)
20	411 \pm 35.3	408 \pm 20.1	403 \pm 26.6	392 \pm 19.3
Lactation				
1	309 \pm 30.6	305 \pm 22.7	297 \pm 19.4	279** \pm 18.5 ($\downarrow 10$)
4	326 \pm 31.3	321 \pm 20.3	315 \pm 19.5	300** \pm 17.1 ($\downarrow 8$)
10	351 \pm 26.7	344 \pm 20.0	337 \pm 20.4	325** \pm 23.6 ($\downarrow 7$)
21	345 \pm 23.0	341 \pm 19.3	336 \pm 21.2	333 \pm 17.6

a Data were obtained from the study report Tables 6 and 8, pages 94 and 96. Percent difference from controls is presented parenthetically; n= 23-25.

* Significantly different from controls at $p \leq 0.05$.

** Significantly different from controls at $p \leq 0.01$.

3. **Reproductive performance:** Pregnancy rate, number of implantations/dam, gestation length, and sex ratio were comparable between treated and control animals (Table 4).

Table 4. Delivery observations in P females administered isoxaflutole from GD 6 to LD 10. ^a

Observation	Dose (mg/kg/day)			
	0	5	25	250
# Animals Mated	25	25	25	25
# Animals Pregnant	23	25	24	24
Pregnancy Rate (%)	(92)	(100)	(96)	(96)
# Nonpregnant	2	0	1	1
Mean (\pm SD) gestation length (days)	21.6 \pm 0.58	21.5 \pm 0.51	21.4 \pm 0.49	21.9 \pm 0.34
Total # Implantations ^b	368	400	355	396
Mean (\pm SD) Implantations/Dam	16.0 \pm 2.51	16.0 \pm 1.86	15.4 \pm 2.55 ^c	16.5 \pm 2.41
Total # of Litters Examined	23	25	24	24
Sex Ratio (% Male, \pm SD)	50.5 \pm 13.07	48.6 \pm 11.50	47.7 \pm 13.22	51.1 \pm 12.92

a Data extracted from the study report Table 1, page 73; Table 14, page 102; Table 17, page 105; Table 18, page 106.

b Calculated by reviewers from data presented in this table.

c n=23

4. Maternal postmortem results

- a. **Macroscopic examination:** No treatment-related pathological abnormalities were observed in any treated group.
- b. **Microscopic examination:** Microscopic examinations were not conducted on adult animals.

B. OFFSPRING

1. **Viability and clinical signs:** No treatment-related differences in live litter size, post-natal survival, or sex ratios were observed in any treated group (Table 5). Decreased ($p \leq 0.05$) survival was observed in the 250 mg/kg pups during PNDs 0-1 only (93.2% treated vs. 98.3% controls). No treatment-related clinical signs were observed.

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- parenthetically; n = 23-89.
- * Significantly different from controls at p≤0.05
- ** Significantly different from controls at p≤0.01

Table 6b. Selected F₁ pup mean (±SD) body weight gains (g).^a

Post-natal Day	Dose (mg/kg/day)			
	0	5	25	250
Males				
1-4	2.8±0.75	2.9±0.70	2.8±0.80	2.2*±0.58 (↓21)
21-28	35.8±7.11	36.3±5.85	35.4±6.51	33.5*±5.19 (↓6)
70-72	9.0±7.59	10.5±4.73	10.3±5.29	8.8±3.94
(Post-weaning) 21-72	374.2±42.02	384.9±38.32	383.8±33.46	359.9±39.56
Females				
1-4	2.6±0.82	2.7±0.70	2.7±0.76	2.2±0.53
4-7	5.3±1.17	5.3±1.21	5.3±0.74	4.5*±0.85 (↓15)
17-21	12.2±2.64	11.9±2.53	10.5±1.76	11.3±2.12
63-70	14.6±6.62	15.3±7.30	18.4*±7.44 (↑26)	18.8**±6.16 (↑29)
70-72	4.0±7.47	4.4±7.04	3.7±7.19	3.1±6.82
(Post-weaning) 21-72	206.8±26.85	208.7±24.14	216.5±26.42	209.4±23.01

- a Data obtained from the study report Table 24, pages 163 through 168. Percent difference from controls is presented parenthetically; n = 23-89.
- * Significantly different from controls at p≤0.05
- ** Significantly different from controls at p≤0.01

3. Developmental landmarks

- a. **Sexual maturation:** A slight delay in mean balanopreputial separation (44.1 days treated vs. 42.6 days controls [↑4%], p≤0.05) was observed in the 250 mg/kg males (Table 7). No differences in vaginal patency were observed between treated and control F₁ females.

the 5 mg/kg females at PND 60 (\downarrow 15%, $p \leq 0.05$) was not dose-dependent and considered unrelated to treatment.

Table 9. Mean (\pm SD) auditory startle reflex data.^a

Observation		Dose (mg/kg/day)			
		0	5	25	250
Males					
PND 20	V _{max} (mv)	161.7 \pm 68.01	193.7 \pm 37.64	168.5 \pm 59.05	161.7 \pm 58.23
	T _{max} (ms)	26.5 \pm 4.67	24.3 \pm 2.12	25.2 \pm 2.35	26.1 \pm 3.26
	V _{ave} (mv)	35.4 \pm 12.45	44.4 \pm 7.29	37.7 \pm 11.89	37.3 \pm 13.90
PND 60	V _{max} (mv)	195.5 \pm 90.87	169.5 \pm 82.87	207.4 \pm 172.51	154.2 \pm 87.72
	T _{max} (ms)	30.3 \pm 4.38	34.4 \pm 5.27	30.7 \pm 4.87	32.4 \pm 3.43
	V _{ave} (mv)	42.7 \pm 18.74	36.7 \pm 15.93	42.3 \pm 31.41	32.8 \pm 17.09
Females					
PND 20	V _{max} (mv)	198.3 \pm 37.67	173.7 \pm 57.69	204.1 \pm 95.33	171.1 \pm 54.79
	T _{max} (ms)	23.2 \pm 2.00	23.2 \pm 2.35	22.7 \pm 2.93	23.3 \pm 3.02
	V _{ave} (mv)	44.4 \pm 8.15	38.9 \pm 10.18	44.1 \pm 20.36	38.0 \pm 11.85
PND 60	V _{max} (mv)	106.8 \pm 56.02	171.7 \pm 119.51	86.2 \pm 41.06	112.4 \pm 59.53
	T _{max} (ms)	33.6 \pm 4.46	28.6 \pm 2.98* (\downarrow 15)	34.5 \pm 5.15	32.7 \pm 4.59
	V _{ave} (mv)	21.5 \pm 10.95	35.1 \pm 23.66	18.6 \pm 9.25	22.4 \pm 11.43

^a Data obtained from Study Report Table 29, pages 175-176; n=10. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).

* Statistically different from control, $p \leq 0.05$

- d. **Learning and memory testing:** No treatment-related differences in swimming ability, learning, or memory were noted in any treated group relative to concurrent controls (Tables 10a and 10b). Mean Day 1 swimming ability (PND 62) was decreased (increased time) relative to concurrent controls in the 250 mg/kg males (7.35 sec. treated vs. 5.76 sec. controls, $p \leq 0.05$). However, in the absence of effects during the actual test (swimming maze), this finding was considered to be incidental.

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Test Day/Parameter		Dose (mg/kg/day)			
		0	5	25	250
PND 62					
Day 1	Swimming ability (sec)	5.76±1.271	6.13±1.251	5.34±0.947	7.35±1.679* (128)
Day 2	Time (sec)	109.81±36.242	95.72±45.942	79.88±48.036	94.58±55.871
Path A Trial 1	Errors	21±7.3	17±11.8	13±8.4	16±8.9
Day 2	Time (sec)	54.15±33.351	51.98±40.280	44.59±28.018	57.84±49.867
Path A Trial 2	Errors	12±8.8	11±7.5	9±6.4	12±10.0
Day 3	Time (sec)	47.32±29.021	30.54±12.127	34.83±35.638	53.17±52.773
Path A Trial 3	Errors	9±7.5	5±2.2	6±5.8	9±10.9
Day 3	Time (sec)	20.36±7.136	23.84±16.064	23.93±24.782	24.93±18.154
Path A Trial 4	Errors	6±3.7	5±4.0	4±6.7	5±6.3
Day 4	Time (sec)	130.87±57.626	152.53±45.189	164.50±41.367	138.45±58.303
Path B Trial 5	Errors	19±9.6	26±11.0	31±10.7	23±11.7
Day 4	Time (sec)	71.09±55.932	109.08±71.494	99.88±66.538	120.90±73.104
Path B Trial 6	Errors	13±10.5	22±16.1	19±14.8	22±14.0
Day 5	Time (sec)	60.22±54.105	93.38±65.872	72.87±49.980	120.40±66.146
Path B Trial 7	Errors	11±11.1	16±11.6	11±10.2	20±15.0
Day 5	Time (sec)	73.33±63.689	44.09±30.979	32.23±31.400	33.47±22.320
Path B Trial 8	Errors	13±11.2	8±7.0	5±6.6	7±7.4
Day 6	Time (sec)	44.86±53.783	21.87±14.776	62.29±53.209	31.65±21.028
Path B Trial 9	Errors	6±9.5	3±3.2	8±8.2	5±5.3
Day 6	Time (sec)	26.47±27.818	15.52±8.072	41.56±35.965	21.14±11.500
Path B Trial 10	Errors	4±7.3	2±2.3	7±8.5	3±2.9
Day 7	Time (sec)	45.94±36.922	71.50±56.031	63.34±48.703	61.05±33.974
Path A (probe) Trial 11	Errors	9±9.1	15±11.3	15±14.3	15±11.3
Day 7	Time (sec)	31.60±18.636	40.01±45.352	57.11±65.432	37.02±17.072
Path A (probe) Trial 12	Errors	7±5.8	6±9.7	11±13.3	9±5.0
Overall Biel (Trials 1-10)	Time (sec)	63.85±22.055	63.87±10.624	66.39±21.309	67.85±20.814
	Errors	11±3.8	12±2.4	11±3.9	13±3.2
Overall probe (Trials 11-12)	Time (sec)	38.77±24.366	55.76±44.162	60.23±47.272	49.04±22.204
	Errors	8±6.7	11±8.5	13±11.7	12±7.2

a Data obtained from Study Report Tables 31, pages 179-182 and 32, pages 187-190; n=10. Path A = forward through maze; Path B = reverse through maze; Time = mean time to escape; Error = all four feet into an incorrect channel.

* Statistically different from control at $p \leq 0.05$

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Test Day/Parameter		Dose (mg/kg/day)			
		0	5	25	250
PND 62					
Day 1	Swimming ability (sec)	8.16±3.360	8.53±1.971	8.65±3.451	7.60±1.779
Day 2	Time (sec)	77.95±60.604	68.62±26.602	66.31±56.166	76.11±50.187
Path A Trial 1	Errors	15±10.9	12±6.1	11±12.6	15±8.5
Day 2	Time (sec)	65.33±58.069	65.83±52.220	57.38±52.689	55.00±42.780
Path A Trial 2	Errors	13±13.6	13±11.1	10±9.4	11±9.2
Day 3	Time (sec)	45.75±33.949	46.25±41.737	37.44±35.504	53.19±34.298
Path A Trial 3	Errors	8±4.2	8±7.4	5±3.4	10±7.5
Day 3	Time (sec)	31.36±22.881	43.68±45.682	28.14±28.722	47.89±41.921
Path A Trial 4	Errors	6±5.7	9±14.1	4±6.1	12±12.6
Day 4	Time (sec)	139.53±67.970	112.73±49.766	133.59±47.187	149.50±56.578
Path B Trial 5	Errors	24±13.8	19±7.6	25±6.7	27±11.6
Day 4	Time (sec)	92.44±61.246	71.04±57.392	91.88±68.531	122.37±62.803
Path B Trial 6	Errors	16±12.8	13±11.6	16±13.8	24±14.1
Day 5	Time (sec)	60.64±46.830	71.07±64.558	75.81±65.797	104.53±73.381
Path B Trial 7	Errors	9±9.2	10±12.4	12±12.5	22±16.8
Day 5	Time (sec)	26.69±16.406	40.71±32.215	45.32±53.622	50.34±54.202
Path B Trial 8	Errors	3±3.4	6±6.3	7±10.3	8±11.2
Day 6	Time (sec)	27.39±13.418	45.42±31.865	38.47±29.906	63.66±66.376
Path B Trial 9	Errors	3±4.2	7±7.0	5±4.8	12±15.3
Day 6	Time (sec)	32.39±16.742	17.34±7.528	31.56±25.776	52.89±47.545
Path B Trial 10	Errors	6±4.2	1±1.6	4±6.1	10±9.9
Day 7	Time (sec)	43.86±44.061	52.50±49.604	42.23±33.559	32.29±17.276
Path A (probe) Trial 11	Errors	9±9.7	9±10.6	7±4.5	6±4.4
Day 7	Time (sec)	46.24±39.342	57.80±50.463	53.24±50.268	33.37±14.727
Path A (probe) Trial 12	Errors	10±12.2	10±12.0	10±14.0	8±8.6
Overall Biel (Trials 1-10)	Time (sec)	59.95±19.748	58.27±20.134	61.79±29.904	77.55±27.998
	Errors	10±3.9	10±3.7	10±4.6	15±5.3
Overall probe (Trials 11-12)	Time (sec)	45.05±36.357	55.15±39.753	47.74±26.359	32.83±9.324
	Errors	10±9.3	10±8.1	8±6.1	7±4.4

a Data obtained from Study Report Tables 31, pages 183-186 and 32, pages 191-194; n=10. Path A = forward through maze; Path B = reverse through maze; Time = mean time to escape; Error = all four feet into an incorrect channel.

* Statistically different from control at $p \leq 0.05$

5. Postmortem results

- a. **Brain weights:** No treatment-related differences in brain weights or dimensions were noted. Absolute brain weight was decreased ($p \leq 0.05$) in the 250 mg/kg males and females on PND 11 (↓11-12%). Relative (to body) brain weight was increased ($p \leq 0.05$) in the 250 mg/kg males on PND 72 (↑10%) while absolute brain weight was unaffected; this increase was considered to be due to decreased ($p \leq 0.05$) terminal body weights in these animals (↓14%). Decreased brain width was noted in the 5 mg/kg males on PND 11 (↓4%, $p \leq 0.05$); however, this decrease was not dose-dependent and therefore unrelated to treatment.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: It was concluded that maternal toxicity at 250 mg/kg/day was characterized by decreased body weight gain and food consumption. Developmental and/or neonatal toxicity at 250 mg/kg/day was characterized by decreased postnatal survival for PND 0-1 and decreased pup body weights. The maternal NOAEL was 25 mg/kg/day. The reproductive NOAEL was 250 mg/kg/day. The developmental neurotoxicity NOAEL was 250 mg/kg/day.

B. REVIEWER'S COMMENTS: No unscheduled parental deaths occurred during the study. Clinical signs, gross pathology, pregnancy rate, number of implantations/dam, gestation length, and sex ratio were unaffected by treatment. Reproductive function was not evaluated.

In the 250 mg/kg dams, body weights were slightly decreased ($p \leq 0.05$) during GDs 9-15 ($\downarrow 4-6\%$) and LDs 1-10 ($\downarrow 7-10\%$). Body weight gains were decreased ($p \leq 0.01$) during GDs 6-9 ($\downarrow 91\%$) and 9-12 ($\downarrow 39\%$). Body weight gains were also decreased for the entire gestation treatment interval (GDs 6-20; $\downarrow 14\%$, $p \leq 0.01$) and overall gestation (GDs 0-20; $\downarrow 11\%$, $p \leq 0.05$). During the lactation treatment interval (LDs 1-10), body weight gains were comparable to controls in all treated groups. Body weight gains were increased ($p \leq 0.01$) during LDs 10-21 ($\uparrow 133\%$) and LDs 1-21 ($\uparrow 46\%$). Absolute (g/animal/day) food consumption was reduced ($p \leq 0.05$) during GDs 6-15 ($\downarrow 9-23\%$), 6-20 ($\downarrow 9\%$), and 0-20 ($\downarrow 9\%$). Absolute food consumption was also decreased during LDs 1-4 ($\downarrow 17\%$, $p \leq 0.01$). Relative (g/kg/day) food consumption was reduced ($p \leq 0.01$) during the GD intervals 6-9 ($\downarrow 19\%$), 9-12 ($\downarrow 14\%$), and 15-20 ($\downarrow 11\%$). However, relative food consumption was increased ($p \leq 0.05$) at 250 mg/kg after treatment during LDs 10-16 ($\uparrow 9\%$) and 10-21 ($\uparrow 6\%$).

The maternal LOAEL is 250 mg/kg/day based on decreased body weights, body weight gains, and food consumption. The maternal NOAEL is 25 mg/kg/day.

No treatment-related differences in live litter size, post-natal survival, sex ratios, clinical signs, or sexual maturation were observed in any treated group. Pup swimming ability, learning, memory, motor activity, auditory startle response, brain weights and dimensions, and neuropathology were unaffected by the test substance.

Body weights at 5 and 25 mg/kg/day were comparable to that of the controls. At 250 mg/kg, body weights were decreased ($p \leq 0.05$) in the males from PND 1 to 28 ($\downarrow 7-12\%$) and from PND 49 to 63 ($\downarrow 4-5\%$). In the females, body weights were decreased ($p \leq 0.05$) sporadically between PND 1 and 35 ($\downarrow 5-12\%$). Decreased ($p \leq 0.05$) body weight gains were observed sporadically in the males between PNDs 1 and 28 ($\downarrow 6-21\%$) and only during PNDs 4-7 in the females ($\downarrow 15\%$). Absolute brain weights were also reduced (11-12%) in males and females on PND 11 at 250 mg/kg/day, an effect likely related to the decreased body weights and body weight gains observed in these animals.

A NOAEL/LOAEL for offspring can not be determined due to the lack of morphometric analyses in this study.