# DATA EVALUATION RECORD

August 22, 2002
Post DNT Committee Draft
BAS 510 F

Study Type: §83-6, Developmental Neurotoxicity Study in Rats

Work Assignment No. 4-02-182 (formerly 4-01-182) MRID 45404907

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## DATA EVALUATION RECORD

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

**PC CODE:** 128008 **DP BARCODE:** D278864
**SUBMISSION NO.:** S604279

**TEST MATERIAL (PURITY):** BAS 510 F (96.3% a.i.)

**SYNONYMS:** 3-Pyridinecarboxamide, 2-chloro-N-(4'- chloro-1,1-biphenylU-2-yl)·(CAS name)
2-chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide (IUPAC name)

**CITATION:** Kaufmann, W., Schilling, K., Mellert W., van Ravenzwaay B. (2001) BAS 510 F; Developmental Neurotoxicity Study in Wistar Rats, Administration in the Diet. Experimental Toxicology and Ecology, BASF, Ludwigshafen, Germany. Laboratory Project Id.: 67R0179/97167, February 8, 2001. MRID 45404907. Unpublished.

**SPONSOR:** BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, NC

**EXECUTIVE SUMMARY:** In a developmental neurotoxicity study (MRID 45404907) BAS 510 F (96.3% a.i., N46) was administered to 35 female Crl: WI (GLX/BRL/HAN) IGS BR (Wistar) rats per dose in the diet at dose levels of 0, 100, 1,000, or 10,000 ppm [0, 14, 147, and 1442 mg/kg/day, average] from gestation day (GD) 6 through lactation day (LD) 21. On postnatal day (PND) 4, litters were standardized to 8 pups or more/litter. Offspring from culled litters (1 male or female pup/litter) were assigned to subgroups for further examination [brain weights, neuropathology (I, III), learning and memory (IV, V), motor activity(II), and auditory startle response(III)] no later than one day before examinations commenced. Pups were weaned on postnatal day 21, after which time maternal animals were killed.

No mortalities, significant treatment-related clinical signs or open field observations, changes in body weight or food consumption, or changes in the duration of gestation, numbers of litters, or intercurrent deaths were noted in maternal animals. The high dose (1442 mg/kg/day) exceeded the limit dose for this study.

**A maternal LOAEL was not observed.**

**The maternal NOAEL is 10,000 ppm [1442 mg/kg/day].**

In pups, no treatment effects on litter size or viability during lactation were seen. In high dose pups prior to weaning, there were significant decreases in body weight in males (↓6-14%; PND 4-12) and in females (↓6-16%; PND 1-21). In 1000 ppm pups, body weight gains were reduced 21% (PND 1-4), and body weights were significantly decreased in males (8%) and females (9%) on PND 4, but recovered by day 11. No effects on postweaning body weights, or the day of preputial separation or vaginal opening were found. In the FOB, increased head shaking among high dose male pups on PND 4, and slightly increased signs of increased activity or urination, or irregular respiration were seen among high dose pups. No consistent effects on motor activity were seen. Statistically significant decreases in acoustic startle reflex amplitudes were noted in males in the high (-19%) and low (-24%) dose groups, while a 15% decrease in mid dose males was not statistically significant. This effect was consistent across all blocks within the session, but greatest in the first block, with statistically significant decreases in the first block in the low (-24%) and mid dose (-32%) groups. Females showed a similar pattern of decreases, but statistical significance was seen only in block 2 in the low (-38%) and mid dose (-24%) groups. No effects on learning and memory performance were seen, but there are concerns about the validity of the procedures. Both sexes of PND 11 pups in the 10,000 ppm group showed statistically significant decreases in body weight (9%) and brain weight(6% - 7%). No changes in microscopic pathology were found. Significant decreases in brain length (3%) in high dose males on PND 11, and in the right hippocampus (8%) of high dose females on PND 11 were found. Left hippocampus measures in this group showed a similar, but non-significant 6% decrease.

**The offspring LOAEL is 100 ppm [14 mg/kg/day], based on decreases in acoustic startle reflex amplitude (24-27%) in both sexes on PND 24.**

**The offspring NOAEL is less than 100 ppm [14 mg/kg/day].**

This study is classified **acceptable/non-guideline** and does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft). The bases for rating this study as non-guideline are the lack of positive control data, limited data on the learning and memory test, and the failure to perform morphometric measurements of the hippocampus in the mid and low dose groups in response to the effect at the high dose level. This study can be re-classified if these deficiencies are adequately addressed.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. A Flagging statement was not provided.

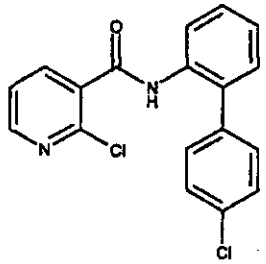
3

# I. MATERIALS AND METHODS

## A. MATERIALS:

**1. Test Material:** BAS 510 F

| | |
|---|---|
| **Description:** | White powder |
| **Lot/Batch #:** | N46 |
| **Purity:** | 96.3% a.i. |
| **Compound Stability:** | The compound was stable in the diet at room temperature for 32 days. |
| **CAS # of TGAI:** | 188425-85-6 |




**2. Vehicle and/or positive control:** Diet

**3. Test animals (P):**

| | | |
|---|---|---|
| **Species:** | Rat | |
| **Strain:** | Crl: WI (GLX/BRL/HAN) IGS BR | |
| **Age at study initiation:** | Age was not provided. | |
| **Group mean wt. at study initiation:** | 171.5-173.7 g on GD 0 | |
| **Source:** | Charles River Laboratories, Sulzfeld, Germany | |
| **Housing:** | Individually, in stainless steel wire-mesh cages; during mating and lactation, in Makrolon type M III cages. | |
| **Diet:** | Kliba maintenance diet rat/mouse/hamster meal (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum* | |
| **Water:** | Tap water, *ad libitum* | |
| **Environmental conditions:** | **Temperature:** | 20-24°C |
| | **Humidity:** | 30-70% |
| | **Air changes:** | Not reported |
| | **Photoperiod:** | 12 hrs dark/ 12 hrs light |
| **Acclimation period:** | 5 days | |

## B. PROCEDURES AND STUDY DESIGN

**1. In life dates** - Start: 4/16/2000 End: 7/18/2000

**2. Study schedule:** The maternal animals were mated and assigned to study. The test substance was administered to the maternal animals from gestation day (GD) 6 through lactation day (LD) 21. Pups were weaned on postnatal day 21, after which time maternal animals were killed. F1 pups were assigned to subgroups in order to evaluate brain weights, neuropathology, learning and memory, motor activity, and auditory startle response (Table 1).

3. **Mating procedure:** Females were paired 1-3:1 with males of the same strain and source. Each female was examined during the mating period to identify sperm in a vaginal smear; the day that sperm was found was designated gestation day 0. Beginning on GD 18, dams were individually housed in nesting boxes, where they were maintained through LD 14.

4. **Animal Assignment:** Mated females were assigned to dose groups as indicated in Table 1. Offspring (1 pup/sex/litter) from culled litters were assigned to testing subgroups no later than one day before examinations commenced (Table 1).

**Table 1. Study design** [a]

| Experimental Parameter | Subgroup | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 100 | 1000 | 10000 |
| **Maternal Animals** | | | | | |
| No. of maternal animals assigned | NA | 35 | 35 | 35 | 35 |
| FOB (open field)<br>(GD 7, 14; LD 7, 14) | NA | 10 | 10 | 10 | 10 |
| **Offspring** | | | | | |
| FOB (open field)<br>(PND 4, 11, 21, 35, 45, 60) | 2 | 10/sex | 10/sex | 10/sex | 10/sex |
| Learning and Memory<br>(PND 21 and 28)<br>(PND 60 and 67) | 4<br>5 | 10/sex<br>10/sex | 10/sex<br>10/sex | 10/sex<br>10/sex | 10/sex<br>10/sex |
| Motor activity<br>(PND 13, 17, 21, 60) | 2 | 10/sex | 10/sex | 10/sex | 10/sex |
| Auditory startle habituation<br>(PND 24 and 60) | 3 | 10/sex | 10/sex | 10/sex | 10/sex |
| Perfusion fixation, brain weights, and neuropathology<br>(PND 11)<br>(PND 60) | 1<br>3 | 10/sex<br>10/sex | 10/sex<br>10/sex | 10/sex<br>10/sex | 10/sex<br>10/sex |

a Adapted from pages 25, 28, and 31 of the study report.

5. **Dose selection rationale:** No dose rationale was provided.

6. **Dosage administration:** All doses were administered to maternal animals continuously in the diet from GD 6 through LD 21. Treated diet was available to pups during lactation, some consumption during the third week of life is likely.

7. **Dosage preparation and analysis:** The test substance was weighed and mixed with a small amount of diet to form a premix. The premix was diluted with additional diet to achieve the desired test concentrations. Stability analyses of the test substance in the diet (100 mg/kg) for up to 32 days at room temperature were performed prior to the study. Homogeneity and

concentration of all dose formulations were determined before the dose administration period and every time a dose formulation was prepared.

**Results - Homogeneity Analysis (range as % of nominal):**

100 ppm: 93.2-101.6%
1000 ppm: 91.7-97.1%
10000 ppm: 93.2-101.6%

**Stability Analysis:** 97.9% of nominal after storage at room temperature for 32 days.

**Concentration Analysis (range as % of nominal):**

100 ppm: 93.2-101.6%
1000 ppm: 91.7-97.1%
10000 ppm: 93.2-101.6%

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

## C. OBSERVATIONS

### 1. In-life observations

**a. Maternal animals:** All animals were evaluated daily for mortality, moribundity, and clinical signs of toxicity. Open field observations were conducted in a standard arena on 10 dams/group on gestation days (GD) 7 and 14 and lactation days (LD) 7 and 14. The following observations were recorded:

| Open field observations | | | |
|---|---|---|---|
| X | Behavior when handling | X | Abnormal movements |
| X | Fur | X | Gait abnormalities |
| X | Skin | X | Lacrimation |
| X | Posture | X | Palpebral closure |
| X | Salivation | X | Exophthalmos |
| X | Respiration | X | Feces (appearace/consistency) |
| X | Activity/arousal level | X | Urine |
| X | Tremors | X | Pupil size |
| X | Convulsions | X | Other abnormalities |

Body weights were recorded on GDs 0, 6, 13, and 20 and LDs 1, 7, 14, and 21. Food consumption was determined on GDs 0, 6, 13, and 20 and LDs 1, 7, and 14.

**b. Offspring:**

1) **Litter observations:** The day of completion of parturition was designated as lactation day (postnatal day, PND) 0. Live pups were counted, sexed and weighed individually for each litter on PNDs 1, 4, 11, 17, and 21. All live offspring were observed daily for clinical signs of toxicity and gross signs of mortality or morbidity.

On PND 4, litters were standardized to at least 8 pups/litter; excess pups were killed and discarded. Offspring (1 pup/sex/litter) from culled litters were assigned to testing subgroups no later than one day before examinations commenced (Table 1).

2) **Developmental landmarks:** Beginning on PND 40, all selected offspring (subgroups 1, 2, 3, and 5) were examined daily for balanopreputial separation. Beginning on postnatal day 27, female offspring (subgroups 1, 2, 3, and 5) were examined daily for vaginal patency. The age of onset was recorded.

3) **Postweaning observations:** Clinical observations were noted daily and body weights were recorded weekly for all animals during the post-weaning period.

4) Neurobehavioral evaluations

i) Functional observational battery (FOB): Open field observations were conducted on 10 pups/sex/group (subgroup 2) on PNDs 4, 11, 21, 35, 45, and 60. The following observations were recorded:

| Open Field Observations | | | |
|---|---|---|---|
| X | Behavior when handling | X | Abnormal movements |
| X | Fur | X | Gait abnormalities |
| X | Skin | X | Lacrimation |
| X | Posture | X | Palpebral closure |
| X | Salivation | X | Exophthalmos |
| X | Respiration | X | Feces (appearance/consistency) |
| X | Activity/arousal level | X | Urine |
| X | Tremors | X | Pupil size |
| X | Convulsions | X | Other abnormalities |

ii) Motor activity testing: Motor activity was evaluated in 10 pup/sex/exposure group (subset 2) on PNDs 13, 17, 21, and 60. The measurement was performed in the dark using the Multi-Varimex-System (Columbus Instruments Int. Corp., OH, USA). Each test session was one hour in duration, and consisted of 12, five-minute intervals. The number of beam interruptions was tabulated.

iii) Auditory startle reflex habituation: Auditory startle reflex habituation testing was performed on 10 pup/sex/exposure group (subset 3) on PNDs 24 and 60 using the SR-LAB; STARTLE RESPONSE SYSTEM (San Diego Instruments, San Diego, CA, USA). Measurements were carried out with the light and ventilator switched on in the measurement chambers. The test sessions consisted of a 5 minute acclimatization period with a 70 db background noise; the startle stimulus was about 120 db. Test sessions consisted of 50 trials with a 5 second inter-trial interval. Response was recorded for 50 ms. Data (maximum amplitude) were analyzed in 5 blocks of 10 trials each.

iv) Learning and memory testing: Watermaze testing was performed on 10 pup/sex/exposure group on PNDs 21 and 28 (subset 4) and 60 and 67 (subset 5). The test maze is a 3 alley "m"

maze 60 cm long, and 100 cm across. Basin height is 29 cm with room temperature water filled to 10 cm below the upper rim. The center alley is the start area and the steps placed on either the left or right alley. The first learning ability test (Learning 1) consisted of 6 trials at an interval of 1 hour for each selected animal. At every trial, the animals had to locate a ladder on the right side of the maze. The maximum duration for the animals to swim was 6 minutes. Successful trials are defined as reaching the exit following a direct path, i.e., without movement in a direction opposite to that of the placement of the steps. After a period of one week, the same animals had to locate the ladder on the right side of the water maze pool again (Memory). The relearning ability test (Learning 2) started one hour after the beginning of the memory test. For this test, the procedure for Learning 1 was followed, except that the animals had to locate the ladder on the left side of the pool. Measures for this test consisted of the number of successful trials out of five for the learning phases, and the % of correct trials/group for the memory test.

2. **Postmortem observations:**

**a. Maternal animals:** All dams were sacrificed after their offspring were weaned. No pathological examination was conducted.

**b. Offspring:** On PND 11 (subgroup 1) or 60 (subgroup 3), ten pups/sex/exposure group (one male and one female per litter) were sacrificed by perfusion fixation. SOERESEN phosphate buffer was used as a rinsing solution and neutrally buffered 4% formaldehyde solution was used as the fixative. Brain weight, length, and maximum width was measured for each perfused animal. The tissues listed below (and all gross lesions) were prepared for examination.

| CENTRAL NERVOUS SYSTEM | | PERIPHERAL NERVOUS SYSTEM | |
|---|---|---|---|
| | **BRAIN** | | **SCIATIC NERVE** |
| X | Frontal lobe | X | Proximal* |
| X | Parietal lobe with diencephalon | | |
| X | Midbrain with occipital and temporal lobe | | |
| X | Cerebellum | | |
| X | Pons | | |
| X | Medulla oblongata | | |
| X | Olfactory bulb | | |
| | **SPINAL CORD** | | **OTHER** |
| X | Cervical swelling (C1-C5) | | Sural Nerve |
| X | Lumbar swelling (L1-L4) | X | Tibial Nerve (proximal and distal)* |
| X | Thoracic swelling (Th5-Th8) | | Peroneal Nerve |
| | | X | Lumbar dorsal root ganglion (L1-L4)* |
| | | X | Lumbar dorsal root fibers (L1-L4)* |
| | **OTHER** | X | Lumbar ventral root fibers (L1-L4)* |
| X | Gasserian ganglia with nerve Trigeminal nerves | X | Cervical dorsal root ganglion (C1-C5)* |
| X | Optic nerve | X | Cervical dorsal root fibers (C1-C5)* |
| X | Eyes | X | Cervical ventral root fibers (C1-C5)* |
| X | Pituitary gland | X | Gastrocnemius muscle |
| X | Nose (nasal cavity) | | |

* Collected and processed on PND 60 (subgroup 3) only.

Tissues from high dose and control animals were embedded in Paraplast, sectioned and stained with hematoxylin & eosin (H&E). The brains (*in toto*) of mid dose animals and the other tissues were preserved in neutrally buffered 4% formaldehyde solution.

Peripheral tissues from day 60 animals (high dose and controls) were subjected to secondary fixation in 5% glutardialdehyde solution, embedded in plastic (epoxy resin) and stained with Azure-II-methylene blue basic-Fuchsin solution (AmbF). Mid dose and control tissues were given secondary fixation in 5% glutardialdehyde solution and the fixed specimens stored in buffered solution.

Only tissues from high dose and control animals were examined.

c. Morphometrics: Morphometry of major brain areas for the same animals selected for neuropathology. Thickness measurements were made on frontal and parietal cortices, caudate/ putamen, hippocampus, corpus callosum, and cerebellum. Bilateral measures were made except for corpus callosum and cerebellum.

## D. DATA ANALYSIS

1. **Statistical analyses:** Statistical significance was denoted at $\alpha$=0.05 or $\alpha$=0.01. Data were analyzed by the following statistical procedures:

| Parameter | Statistical test |
|---|---|
| Food consumption in dams<br>Body weight in dams<br>Body weight gain in dams<br>Body weight in offspring (litter means)<br>Body weight gain in offspring (litter means)<br>Duration of gestation<br>Number of offspring delivered per litter | Dunnett's test (two-sided) |
| Female fertility index<br>Gestation index<br>Dams with liveborn offspring<br>Dams with stillborn offspring<br>Dams with all stillborn offspring<br>Live birth index<br>Stillborn offspring<br>Dead offspring<br>Cannibalized offspring<br>Sacrificed moribund offspring<br>Viability index<br>Lactation index<br>Water maze evaluation | Fisher's Exact test |
| Water maze evaluation | Wilcoxon test (one-sided) |
| Motor activity<br>Startle response | Kruskal-Wallis (two-sided, non-parametric) followed by the Mann-Whitney U test (two-sided, non-parametric) as necessary |
| Brain weights | Kruskal-Wallis (two-sided, non-parametric) followed by pairwise comparison with the Wilcoxon test (two-sided) |
| Brain measurements | pairwise comparison with the Wilcoxon test (one-sided) with Bonferoni-Holm adjustment for whole brain only |

2. **Indices:**

a. **Reproductive indices:** The following reproductive indices were calculated from breeding and parturition records of animals in the study:

**Female fertility index (%) = number of pregnant females / number of mated females x 100**

**Gestation index (%) = number of females with live offspring on LD 0/number of pregnant females x 100**

**Live birth index (%) = number of liveborn offspring / total number born x 100**

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

**Viability index (%) = number of live pups on PND 4 (preculling)/number of liveborn pups on PND 0 x 100**

**Lactation index (%) = number of live pups on PND 21/number of live pups on PND 4 (postculling) x 100**

**Sex ratio (PND 0 or 21) = number of live male or female offspring on PND 0 or 21 / number of live male and female offspring on PND 0 or 21 x 100**

3. Positive control data: No positive control data have been provided. However, this data is currently being collected and is scheduled for completion in 12/02.

## II. RESULTS

### A. MATERNAL ANIMALS

1. **Mortality and clinical and functional observations:** No mortalities occurred in the maternal animals. Furthermore, no significant treatment-related clinical signs or open field observations were noted.

2. **Body weight and food consumption:** Selected group mean body weights, body weight gains, and food consumption values for pregnant or nursing dams are summarized in Table 2. No toxicologically significant treatment-related findings were noted.

Increased ($p \leq 0.05$) body weight gains were observed in the 10,000 ppm females on GDs 13-20 (↑28%) and LDs 7-14 (↑72%); however, these increases were not considered to be toxicologically significant.

Decreased ($p \leq 0.05$) body weight gains were noted in the 1,000 ppm dams on GDs 14-21 (↓81%) and for the entire lactation period (↓20%); however, these decreases were not dose-dependent and were concluded not to be related to treatment.

Sporadic differences ($p \leq 0.05$) in food consumption were noted during gestation (↑10-19%) and lactation (↓6%), but were concluded not to be related to treatment.

**TABLE 2. Mean (±SD) maternal body weight and food consumption [a]**

| Observations/study week | Dose (ppm) | | | |
|---|---|---|---|---|
| | Control | 100 | 1000 | 10000 |
| **Gestation** | | | | |
| Mean body weight (g)<br>GD 0<br>GD 20 | <br>171.6±8.88<br>258.4±35.15 | <br>173.7±8.01<br>265.3±30.92 | <br>171.5±7.33<br>266.0±28.56 | <br>171.6±9.75<br>271.6±26.85 |
| Mean weight gain (g)<br>GD 6-20 | 65.2±31.14 | 71.1±29.00 | 72.7±28.28 | 77.9±22.97 |
| Mean food consumption (g/animal/day)<br>GD 6-20 | 19.4±0.37 | 19.6±0.50 | 22.3±1.62 | 21.2±3.09 |
| **Lactation** | | | | |
| Mean body weight (g)<br>LD 1<br>LD 21 | <br>209.0±12.62<br>252.0±17.32 | <br>206.0±12.41<br>249.0±17.06 | <br>212.1±12.39<br>246.3±13.96 | <br>208.2±13.89<br>256.9±14.27 |
| Mean weight gain (g)<br>LD 1-21 | 43.2±13.46 | 43.0±12.95 | 34.6±7.71* (120) | 47.3±10.21 |
| Mean food consumption (g/animal/day)<br>LD 1-14 | 42.4±11.86 | 40.4±10.57 | 41.6±9.50 | 40.7±10.06 |

a Data obtained from Tables IA-003 through IA-008 on pages 73 through 78 in the study report. Percent difference from controls is presented parenthetically.
n = 22-35
* Statistically different from control, $p \leq 0.05$.

3. Test Substance Intake: Based on maternal food consumption, body weight and dietary analyses, the achieved doses, expressed as mean daily mg test substance/kg body weight during the gestation and lactation periods, are presented in Table 3.

**TABLE 3. Mean maternal test substance intake (mg/kg body weight/day) [a]**

| Period | Dose (ppm) | | |
|---|---|---|---|
| | 100 | 1000 | 10000 |
| **Gestation** | | | |
| Gestation days 6-20 | 9.6±0.49 | 108.6±1.11 | 1031.6±71.55 |
| **Lactation** | | | |
| Lactation days 1-14 | 18.3±3.33 | 186.0±30.42 | 1853.1±342.04 |
| Average | 14 | 147 | 1442 |

a Data obtained from Tables IA-009 and IA-010, pages 79 and 80 in the study report.

4. **Reproductive performance:** Reproductive performance appeared to be unaffected by the test substance (Table 4).

**TABLE 4. Reproductive performance [a]**

| Observation | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 100 | 1000 | 10000 |
| Number mated | 35 | 35 | 35 | 35 |
| Number of litters | 28 | 30 | 30 | 33 |
| Intercurrent deaths | 0 | 0 | 0 | 0 |
| Mean (±SD) gestation duration (days) | 21.8±0.39 | 21.8±0.55 | 21.6±0.49 | 21.7±0.48 |
| Incidence of dystocia | NR | NR | NR | NR |

a Data obtained from pages Tables IA-011 and IA-012 on pages 81 and 82 in the study report.
NR Not reported

5. **Maternal postmortem results:** Pathological examinations were not performed on the dams.

## B. OFFSPRING

1. **Viability and clinical signs:** Litter size and viability results from pups during lactation are summarized in Table 5. No treatment-related effects were noted. In addition, clinical signs were comparable between treated animals and controls.

**TABLE 5. Litter size and viability [a]**

| Observation | Dose (ppm) | | | |
|---|---|---|---|---|
| | Control | 100 | 1000 | 10000 |
| Total number born | 264 | 310 | 288 | 339 |
| Number born live | 263 | 304 | 288 | 337 |
| Number born dead | 1 | 6 | 0 | 2 |
| Sex Ratio Day 0 - % ♂ | 46.0 | 46.7 | 49.0 | 54.0 |
| Deaths, Days 1-4 | 4 | 0 | 0 | 2 |
| Deaths, Days 5-21 | 0 | 2 | 0 | 2 |
| Mean litter size: | | | | |
| Day 0 | 9.4±2.48 | 10.1±1.50 | 9.6±1.71 | 10.2±1.63 |
| Day 4 [c] | 7.6±4.41 | 9.1±3.41 | 8.7±3.40 | 10.2±1.62 |
| Day 4 [d] | 7.6±4.41 | 7.2±2.44 | 7.1±2.43 | 7.9±0.55 |
| Day 11 | 7.6±4.41 | 6.9±2.75 | 6.4±3.25 | 7.5±1.94 |
| Day 17 | 6.9±4.01 | 6.2±2.52 | 5.7±2.94 | 6.9±1.85 |
| Day 21 | 6.9±4.01 | 6.2±2.52 | 5.7±2.94 | 6.8±1.86 |
| Live birth index - % | 100 | 98 | 100 | 99 |
| Viability index - % [e] | 98.2 | 100 | 100 | 99.4 |
| Lactation index - % | 100 | 99 | 100 | 99.2 |

a Data obtained from Tables IA-012 through IA-014, pages 82 through 84 in the study report.
c Before standardization (culling). Litter size changes reflect culling of extra litters between day 1 and 4. This was also done between days 4 and 21.
d After standardization (culling).
e Calculated by the reviewers from data presented in Table IA-012 through IA-014, pages 82 through 84 by dividing the number of pups on PND 4 (precull) by the number of live pups on PND 1 (minus any total litters with scheduled sacrifices), and multiplying by 100.

2. **Body weight:** Offspring preweaning body weights are shown in Table 6a. There were decreases ($p \leq 0.05$) in the 10,000 ppm males between PNDs 4 and 21 (↓6-14%) and in the 10,000 ppm females between PNDs 1 and 21 (↓6-16%). In addition, body weights in 1000 ppm pups were significantly decreased by 8% in males and 9% in females on PND 4, but recovered by day 11. This represents a 21% decrease in body weight gain for both sexes. While significant increases were seen on PND17 in 100 ppm males (8%) and females (7%), which for males persisted into the first post weaning week (Table 6b),these were not considered toxicologically significant. No other treatment-related differences in preweaning or post-weaning body weights were noted.

**TABLE 6a. Mean (±SD) pre-weaning pup body weights (g) [a]**

| Postnatal Day | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 100 | 1000 | 10000 |
| **Males** | | | | |
| 1 | 6.4±0.49 | 6.2±0.59 | 6.3±0.49 | 6.1±0.56 |
| 4 [b] | 9.8±0.85 | 9.3±1.09 | 9.0±0.85* (↓8) | 8.4±0.86** (↓14) |
| 4 [c] | 9.8±0.85 | 9.3±1.11 | 9.0±0.83* (↓8) | 8.4±0.86** (↓14) |
| 11 | 21.5±2.03 | 22.6±1.75 | 21.1±1.43 | 19.6±1.46** (↓9) |
| 17 | 34.4±3.47 | 37.0±2.65** (↑8) | 35.6±2.59 | 32.3±2.31* (↓6) |
| 21 | 47.1±4.31 | 49.3±3.85 | 48.0±3.89 | 43.6±3.18** (↓7) |
| **Females** | | | | |
| 1 | 6.2±0.50 | 5.9±0.52 | 6.0±0.47 | 5.8±0.59* (↓6) |
| 4 [b] | 9.6±0.82 | 9.1±1.06 | 8.7±0.84** (↓9) | 8.1±0.86** (↓16) |
| 4 [c] | 9.6±0.82 | 9.1±1.06 | 8.7±0.83** (↓9) | 8.1±0.84** (↓16) |
| 11 | 21.2±1.97 | 22.1±1.57 | 20.5±1.52 | 19.1±1.35** (↓10) |
| 17 | 33.7±3.27 | 36.0±2.29** (↑7) | 34.4±2.56 | 31.5±2.12** (↓7) |
| 21 | 45.6±4.06 | 47.6±2.68 | 46.1±3.43 | 42.5±2.86** (↓7) |

a Data obtained from Tables IA-015 and IA-016, pages 85 and 86 in the study report. Percent difference from controls is presented parenthetically.

b Before standardization (culling). Litters were sacrificed between days 1-4 and 4-21; thus data are not strictly comparable across days, but are comparable for any one day.

c After standardization (culling).

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

** Statistically different from control, $p \leq 0.01$

**TABLE 6b. Mean (±SD) post-weaning pup body weights (g) [a]**

| Postnatal Week | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 100 | 1000 | 10000 |
| **Males** | | | | |
| 0 | 49.7±4.36 | 55.4±3.38* (111) | 53.7±5.34 | 50.3±4.88 |
| 1 | 87.2±7.53 | 94.6±6.94 | 92.0±8.05 | 88.7±7.76 |
| 2 | 133.0±9.67 | 141.8±13.15 | 139.8±9.84 | 135.9±10.74 |
| 3 | 173.9±12.01 | 181.3±15.84 | 177.6±12.68 | 176.7±11.20 |
| 4 | 214.3±15.12 | 219.7±19.84 | 215.8±14.88 | 216.0±15.01 |
| 5 | 254.3±16.04 | 258.7±21.18 | 253.7±15.30 | 254.8±17.18 |
| **Females** | | | | |
| 0 | 49.4±4.91 | 54.4±3.31 | 49.3±5.30 | 47.0±6.09 |
| 1 | 81.8±6.96 | 87.9±5.97 | 82.6±7.85 | 78.2±7.90 |
| 2 | 114.7±7.49 | 120.3±7.40 | 115.8±8.67 | 111.7±9.54 |
| 3 | 133.6±8.10 | 142.9±9.07 | 134.8±9.97 | 134.0±13.38 |
| 4 | 149.1±10.76 | 159.8±11.59 | 151.3±12.66 | 151.8±14.06 |
| 5 | 164.3±13.86 | 173.7±14.36 | 163.5±15.71 | 167.4±16.80 |

a Data obtained from pages Tables IA-037 and IA-038, pages 107 and 108 in the study report.
* Statistically different from control, $p \leq 0.05$

## 3. Developmental landmarks

**a) Sexual maturation:** Sexual maturation data are presented in Table 7. The numbers of days to preputial separation and vaginal opening were comparable between treated animals and controls.

**TABLE 7. Mean (±SD) age of sexual maturation (days) [a]**

| Parameter | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 100 | 1000 | 10000 |
| N (M/F) | 30/30 | 29/30 | 30/30 | 30/30 |
| Preputial separation (males) | 43.4±1.38 | 43.0±1.73 | 44.2±1.90 | 43.3±1.14 |
| Vaginal opening (females) | 32.5±1.59 | 32.2±1.87 | 32.8±2.27 | 32.8±2.35 |

a Data obtained from Tables IA-019 and IA-020, pages 89 and 90 in the study report.

b) **Physical landmarks:** Physical landmarks were not evaluated.

4. **Behavioral assessments**

a) **Functional observational battery:**

At the high dose, an slightly increased incidence of several effects were seen in male pups: head shaking on PND4, increase in urination on PND 11, increases in slight hyperactivity on PND 21 and 35, with simultaneous (and paradoxical) increases in reduced activity on PND 35. At the same dose, female pups on PND 11 showed increases in irregular respiration and urination. These effects are tabulated in Table 8. Only the increased head shaking was statistically significant by reviewer's analysis (no statistics provided by study authors).

**Table 8. Functional Observational Battery Results (incidence) [a]**

| Observation | Dose (ppm) | | | |
|---|---|---|---|---|
| | Control | 100 | 1000 | 10,000 |
| **Males** | | | | |
| PND 4: Head Shaking | 0 | 2 | 2 | 4* |
| PND 11: Urination | 2 | 3 | 2 | 5 |
| PND 21: increase in slight hyperactivity | 1 | 3 | 1 | 5 |
| PND 35: increase in slight hyperactivity | 1 | 0 | 1 | 4 |
| increase in slightly reduced activity | 1 | 0 | 2 | 4 |
| **Females** | | | | |
| PND 11: Irregular respiration | 2 | 2 | 3 | 5 |
| Increased urination | 2 | 1 | 1 | 5 |

a Data obtained from pages 129-140 in the study report.
N = 10/sex/dose
* Statistically different from control, $p<0.05$; one tailed Fisher's Exact Test, calculated by reviewer.
** Statistically different from control, $p<0.01$

b) Motor activity: Motor activity data are presented in Table 9.

No consistent differences were seen for male or female pups. In males, the largest differences seen were a 46% increase in 1000 ppm males on PND 13, and a 41% decrease in the 100 ppm males on PND 17. In females, the largest difference was a 39% increase in 10,000 ppm females on PND 17. None of these changes were reported as statistically significantly different from controls. The increase in females at 10,000 ppm was seen as less habituation in the later intervals, and could be regarded as a suggestive effect. Statistically significant differences in two within session intervals were noted: in 1000 ppm males on PND 13 (increase), and in 1000 ppm females on PND 60 (decrease). Pre-weaning coefficients of variation ranged from 62% on PND 13 to 36% on PND 21 in males, and from 43% on PND 13 to 38% on PND 21 in females. PND 60 C.V.s were 25% and 19% for males and females respectively.

TABLE 9. Mean (±S.D.) motor activity data (total beam interruptions) [a]

| Test Day | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | C.V. | 100 | 1000 | 10000 |
| **Males** | | | | | |
| PND 13 | 100.3±62.3 | 62% | 88.7±41.6 | 146.0±100.6 (46%) | 97.3±48.7 |
| PND 17 | 216.8±96.0 | 44% | 127.6±81.9 (-41%) | 190.1±140.1 | 230.7±114.5 |
| PND 21 | 166.9±59.4 | 36% | 173.7±44.8 | 134.6±57.5 | 206.6±76.1 |
| PND 60 | 437.4±109.3 | 25% | 420.5±83.6 | 449.7±54.0 | 418.7±49.4 |
| **Females** | | | | | |
| PND 13 | 100.2±42.6 | 43% | 74.7±35.5 | 95.3±42.2 | 120.2±50.3 |
| PND 17 | 170.0±66.9 | 39% | 178.2±108.8 | 140.8±79.7 | 235.7±124.6 (39%) |
| PND 21 | 196.9±74.4 | 38% | 152.9±61.2 | 143.4±43.8 | 197.5±84.9 |
| PND 60 | 365.3±69.3 | 19% | 423.6±71.2 | 410.7±65.9 | 421.8±66.2 |

a Data obtained from Tables IB-001 through IB-016, pages 153 through 168 in the study report.
n = 10

c) **Auditory startle reflex habituation:** Auditory startle reflex data are presented in Table 10.

Statistically significant decreases in amplitude were noted in males in the high(-19%) and low (-24%)dose groups, while a 15% decrease in mid dose group was not statistically significant. This effect was consistent across all blocks within the session, but greatest in the first block, with statistically significant decreases in the first block in the low (-24%)and mid dose (-32%) groups. Females showed a similar pattern of decreases, but statistical significance was seen only in block 2 in the low (-38%) and mid dose (-24%) groups. Coefficients of variation in controls on PND 24 were 18% in males,and 30% in females. There is also a lack of a dose response for this effect. But despite these limitations, it is concluded that the effects should be regarded as treatment related at all doses because it is broadly consistent and in both sexes.

On PND 60, an isolated 55% increase in block 2 of the low dose females was noted. A 21% decrease in 10,000 ppm females was not statistically significant. No other changes were noted at PND 60. Coefficients of variation in controls on PND 60 were 46% in males,and 41% in females.

**TABLE 10. Auditory Startle Reflex Maximum Amplitude Data (mean ±S.D.) [a]**

| Block | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 100 | 1000 | 10000 |
| **Males** | | | | | |
| PND 24 | 1 | 411.1±102.4 | 280.1±70.9** (-32%) | 304.1±93.5* (-26%) | 322.3±114.1 (-22%) |
| | 2 | 397.0±101.7 | 303.1±105.3 | 347.9±156.2 | 285.5±68.0 |
| | 3 | 390.7±99.8 | 282.7±108.3 | 290.0±97.1 | 313.7±80.2 |
| | 4 | 373.1±96.5 | 304.4±104.8 | 356.0±71.8 | 332.7±83.4 |
| | 5 | 378.8±68.9 | 323.0±131.4 | 354.6±97.9 | 315.9±80.4 |
| | Mean | 390.1±70.2 | 298.7±86.1** (-24%) | 330.5±76.6 (- 15%) | 314.0±73.4* (- 19%) |
| PND 60 | 1 | 872.5±357.6 | 772.9±191.2 | 878.8±354.3 | 863.4±412.4 |
| | 2 | 628.6±440.5 | 482.5±189.6 | 696.4±255.2 | 618.1±250.7 |
| | 3 | 542.5±368.3 | 322.4±156.5 | 528.7±209.2 | 550.7±152.9 |
| | 4 | 512.8±253.8 | 341.6±131.2 | 457.8±144.3 | 529.9±227.3 |
| | 5 | 454.3±212.1 | 349.2±146.6 | 474.1±171.0 | 501.3±243.4 |
| | Mean | 602.1±276.7 | 453.7±135.1 | 607.2±164.4 | 612.7±210.8 |
| **Females** | | | | | |
| PND 24 | 1 | 416.5±116.7 | 353.2±117.8 | 348.4±101.2 | 385.2±139.6 |
| | 2 | 433.2±160.6 | 266.7±82.8* (-38%) | 329.4±84.9* (-24%) | 397.4±180.3 (-8%) |
| | 3 | 395.4±138.2 | 263.5±94.4 | 364.6±162.5 | 319.8±104.4 |
| | 4 | 420.2±172.2 | 289.7±110.9 | 360.6±145.8 | 289.9±72.8 |
| | 5 | 387.0±133.4 | 323.4±136.9 | 399.0±150.6 | 328.3±85.7 |
| | Mean | 410.4±122.4 | 299.3±91.7 (- 27%) | 360.4±115.6 (-12%) | 344.1±94.8 (-16%) |
| PND 60 | 1 | 737.8±416.0 | 756.3±287.4 | 709.7±301.5 | 557.1±151.8 |
| | 2 | 503.0±316.2 | 782.4±600.5 (55%) | 617.3±389.1 | 458.6±128.3 |
| | 3 | 408.8±130.2 | 529.2±231.9 | 455.8±194.6 | 327.5±97.6 |
| | 4 | 419.6±111.6 | 448.4±231.7 | 396.1±160.8 | 321.2±97.8 |
| | 5 | 435.3±165.7 | 446.3±231.9 | 398.4±213.8 | 305.3±105.1 |
| | Mean | 500.9±203.9 | 592.5±277.4 | 515.4±226.2 | 393.9±92.4 (-21%) |

a Data obtained from Tables IB-017 through IB-020, pages 169 through 172 in the study report.
n = 9-10
* Significantly different from controls at $p \leq 0.05$.
** Significantly different from controls at $p \leq 0.01$.

d) Learning and memory testing:

Watermaze performance data are presented in Table 11. Note that data from only the last 5 trials for each session (out of 6) are presented and averaged. No effects on learning and memory performance were seen. The only statistically significant finding was a decrease in the number of successful trials for learning 2 trials for females in the 1000 ppm group. This appears to be an isolated finding, probably unrelated to treatment.

**TABLE 11. Water maze performance (mean ± S.D.) a**

| Session/Parameter | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 100 | 1000 | 10000 |
| **Males** | | | | | |
| Subset 4 PND 21 | Learning 1 | 3.3±1.57 | 3.2±1.55 | 3.6±1.17 | 2.8±1.87 |
| | Memory | 60 % | 50 % | 80 % | 100 % |
| | Learning 2 | 2.4±2.07 | 2.4±1.90 | 2.3±1.89 | 2.4±143 |
| Subset 5 PND 60 | Learning 1 | 3.6±1.35 | 4.1±1.29 | 3.4±1.78 | 4.1±0.74 |
| | Memory | 90 % | 90 % | 80 % | 90 % |
| | Learning 2 | 3.6±1.17 | 2.8±1.62 | 3.2±1.55 | 2.7±1.64 |
| **Females** | | | | | |
| Subset 4 PND 21 | Learning 1 | 3.2±1.40 | 3.6±1.43 | 3.5±1.43 | 3.1±0.88 |
| | Memory | 70 % | 60 % | 80 % | 80 % |
| | Learning 2 | 2.3±1.25 | 2.3±1.57 | 1.2±1.14* | 2.3±1.34 |
| Subset 5 PND 60 | Learning 1 | 3.7±1.25 | 4.5±0.71 | 4.1±1.29 | 4.3±0.95 |
| | Memory | 80 % | 90 % | 80 % | 90 % |
| | Learning 2 | 1.8±1.55 | 1.8±1.87 | 1.8±2.10 | 2.0±2.00 |

a Data obtained from Tables IA-053 and IA-054, pages 123 and 124 in the study report. Data for learning 1 & 2 are number of successful trials, i.e. finding the ladder within 6 mins; memory is percentage of rats who find the ladder within 6 mins. N = 10

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

## 5. Postmortem results

a) **Brain weights:** Brain weights are presented in Table 12. Both sexes of PND 11 pups in the 10,000 ppm group showed statistically significant decreases in body weight (9%) and brain weight(6% - 7%). An 8% increase in body weight in 100 ppm females on PND 11 was not considered toxicologically significant, as were low and mid dose decreases in relative brain weights.

**TABLE 12. Mean (±SD) brain weights [a]**

| Parameter | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 100 | 1000 | 10000 |
| **Males** | | | | |
| **PND 11** | | | | |
| Terminal body weight (g) | 21.77±2.398 | 22.71±1.912 | 21.41±1.519 | 19.81±1.606* (↓9) |
| Absolute brain weight (g) | 1.299±0.082 | 1.287±0.066 | 1.242±0.071 | 1.215±0.072* (↓6) |
| Relative brain weight (%) | 5.997±0.437 | 5.712±0.586 | 5.812±0.252 | 6.15±0.471 |
| **PND 60** | | | | |
| Terminal body weight (g) | 264.99±25.568 | 260.42±20.688 | 264.72±21.075 | 258.24±22.667 |
| Absolute brain weight (g) | 2.02±0.075 | 2.043±0.041 | 2.058±0.071 | 1.976±0.063 |
| Relative brain weight (%) | 0.768±0.062 | 0.788±0.061 | 0.781±0.054 | 0.77±0.062 |
| **Females** | | | | |
| **PND 11** | | | | |
| Terminal body weight (g) | 20.92±1.742 | 22.69±1.835* (↑8) | 21.2±1.621 | 19.03±1.596* (↓9) |
| Absolute brain weight (g) | 1.267±0.054 | 1.231±0.06 | 1.205±0.087 | 1.179±0.049** (↓7) |
| Relative brain weight (%) | 6.07±0.34 | 5.443±0.21** (↓10) | 5.692±0.263* (↓6) | 6.235±0.411 |
| **PND 60** | | | | |
| Terminal body weight (g) | 172.09±12.854 | 170.18±14.789 | 173.1±9.812 | 168.21±11.368 |
| Absolute brain weight (g) | 1.907±0.063 | 1.899±0.035 | 1.928±0.036 | 1.866±0.067 |
| Relative brain weight (%) | 1.111±0.055 | 1.121±0.088 | 1.116±0.056 | 1.113±0.062 |

a Data obtained from Tables IC-1 through IC-8, pages 173 through 180 in the study report. Percent difference from controls is presented parenthetically.
n=10
* Statistically different from control, $p \leq 0.05$
** Statistically different from control, $p \leq 0.01$

**b) Neuropathology**

1) **Macroscopic examination:** No treatment related effects were seen.

2) **Microscopic examination:** No treatment related effects were seen.

3) **Morphometric Measurements**

Offspring morphometric measurements are shown in Table 13. Significant decreases in brain length (3%) in high dose males on PND 11, and in the right hippocampus (8%) of high dose females on PND 11 were found. Left hippocampus measures in this group showed a non-significant 6% decrease. The mid dose and low dose groups (and controls) should be examined to assess any changes in the hippocampus measurements in those female groups. A significant decrease (1%) in brain width was also seen in high dose females on PND 60.

TABLE 13. Morphometric measurements in offspring [a]

| Parameter | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 100 | 1000 | 10000 |
| **PND 11** | | | | |
| Brain length (cm), males | 1.711±0.052 | 1.708±0.030 | 1.676±0.039 | 1.658±0.038* (↓3) |
| Hippocampus, left (µm), females | 1174±104 | NR | NR | 1101±127 (↓6) |
| Hippocampus, right (µm), females | 1207±103 | NR | NR | 1111±105* (↓8) |
| **PND 60** | | | | |
| Brain width (cm), females | 1.522±0.016 | 1.508±0.016 | 1.524±0.016 | 1.502±0.017* (↓1%) |

a Data obtained from Tables IC-013 through IC-020, pages 185 through 192 in the study report. Percent difference from controls is presented parenthetically.
n=8-10; NR = not recorded
* Statistically different from control, $p \leq 0.05$

## III. DISCUSSION and CONCLUSIONS

**A. INVESTIGATORS' CONCLUSIONS:** Administration of BAS 510 F in the diet did not cause maternal toxicity at doses up to 10000 ppm. In the offspring, test substance administration resulted in decreased body weights and body weight gains at 1000 and 10000 ppm, and decreased brain weight and length at 10000 ppm. Clinical and pathological examinations detected no signs of developmental neurotoxicity in the offspring at any dose tested. Therefore, it was concluded that the NOAEL for developmental neurotoxicity was 10000 ppm.

**B. REVIEWER COMMENTS**

**1. PARENTAL ANIMALS:** No treatment-related findings were noted at any dose tested, up to 10,000 ppm (1442 mg/kg/day). Since, however, this dose level is above the limit dose for this type of study, the high dose is considered adequate.

**A maternal LOAEL was not observed.**
**The maternal NOAEL is 10000 ppm (1442 mg/kg/day).**

**2. OFFSPRING:**

In pups, no treatment effects on litter size or viability during lactation were seen. In high dose pups prior to weaning, there were significant decreases in body weight in males(↓6-14%; PND 4-12) and in females (↓6-16%; PND 1-21). In 1000 ppm pups, body weight gains were reduced 21% (PND 1-4), and body weights were significantly decreased in males (8%) and females (9%) on PND 4, but recovered by day 11. No effects on postweaning body weights, or the day of preputial separation or vaginal opening were found.

In the FOB, increased head shaking among high dose males pups on PND 4, and slightly increased signs of increased activity or urination, or irregular respiration were seen among high dose pups. The lack of statistical analyses of these data in the report made their interpretation more difficult, but all of the changes noted occurred at the high dose, and so would not substantively alter the conclusions here.

No consistent effects on motor activity were seen. A 39% increase in motor activity in high dose females on PND 17 was found that was not statistically significant. This increase consisted of less habituation in the later intervals, and could be regarded as a suggestive effect. The pre-weaning coefficient of variation for PND 17 females was 39%, so, the 39% increase approached statistical significance. However, other changes of this magnitude were seen, i.e., a 46% increase in 1000 ppm males on PND 13, and a 41% decrease in the 100 ppm males on PND 17. In summary, this effect was not considered treatment related.

Statistically significant decreases in acoustic startle reflex amplitudes were noted in males in the high (-19%) and low (-24%) dose groups, while a 15% decrease in mid dose group was not

statistically significant. This effect was consistent across all blocks within the session, but greatest in the first block, with statistically significant decreases in the first block in the low (-24%)and mid dose (-32%) groups. Females showed a similar pattern of decreases, but statistical significance was seen only in block 2 in the low (-38%) and mid dose (-24%) groups. There also is little dose response in this data; for both sexes, the peak effect is at the low dose, with about half that response at the mid and high doses. In the absence of historical control or positive control data, there is no comparative basis on which to evaluate these findings. In total, despite these limitations, it is concluded that the effects should be regarded as treatment related at all doses.

Overall, no effects on learning and memory performance were seen. However, reacquisition of the task among females in session 2 (subset V) was poor in all dose groups. In the learning 2 trials the ladder is placed on the other side of the maze. The data indicates that, on average less than 40% of the trials were successful, and all dose groups contain several rats that never have a correct trial in 6 attempts. Successful trials are defined as reaching the exit following a direct path, i.e., without movement in a direction opposite to that of the placement of the steps. Given such a definition, in this situation, where many subjects fail to perform perfectly, it would be more informative to know precisely how these errors are defined, e.g., whole body in the indirect alley, any part of the body, etc., and to know how many errors might have been committed on any one trial. The latency to escape would be a much more informative measure than simply the percentage of successful trials. These two measures would help to better describe differences in the behavior of animals that may have always turned the wrong way, but then corrected themselves, or have swum the right way but been considerably faster or slower in doing so. These differentiations could also help in trying to explain differences in terms of motor or cognitive deficits and to validate this procedure as a learning and memory test. If these are available, they should be provided.

Both sexes of PND 11 pups in the 10,000 ppm group showed statistically significant decreases in body weight (9%) and brain weight (6%-7%). Significant decreases in brain length (3%) in high dose males on PND 11, and in the right hippocampus (8%) of high dose females on PND 11 were found. Left hippocampus measures in this group showed a similar, but non-significant 6% decrease. The hippocampi from the females in the mid dose and low dose groups (and controls) should be examined to assess any changes in the hippocampal measurements in those groups and to establish an NOAEL for this effect, as is called for in the guidelines.

It has been shown in other studies for this chemical that the thyroid is a target. Specifically, a 4 week feeding study (MRID 45404903) reported that exposure of Wistar rats to 15,000 ppm for 4 weeks lead to decreases in thyroid hormones (T3, T4) and increases in TSH. Changes were noted as early as two days after onset of dosing. Because this dose was 50% higher than the high dose of 10000 ppm in the present study, and because no other doses were evaluated, it is difficult to correlate any of the effects seen here with changes in maternal or fetal thyroid function. Goldey et al. (1995) exposed dams from GD 18 to PND21 to 25 ppm of propylthiouracil (PTU, an agent known to target the thyroid) in drinking water. They found persistent decreases in body weights, decreased and/or delayed pre-weaning motor activity, and

persistent post weaning hyperactivity (PND 30-120), decreased acoustic startle amplitudes on PND 24, and increased amplitudes later in life (PND 70). At this dose both decreased T3 and T4 levels were seen. For BASF 510 F, changes in motor activity were inconsistent as noted above, but there were decreases in the startle amplitude. No increases in startle amplitude were seen here at PND 60, but it is not totally comparable to the later testing at PND 70 and later in the published study.

**The offspring LOAEL is 100 ppm [14 mg/kg/day], based on decreases in acoustic startle reflex amplitude (24%) in males on PND 24.**

**The offspring NOAEL is less than 100 ppm [14 mg/kg/day].**

This study is classified **acceptable/non-guideline** and does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft).

C. STUDY DEFICIENCIES:

The bases for rating this study as non-guideline are the lack of positive control data, limited data on the learning and memory test, and the failure to perform morphometric measurements of the hippocampus in the mid and low dose groups in response to the effect at the high dose level. Thus, the study may be upgraded on submission of adequate data to address these issues.

Minor deficiencies were that the age of the dams was not reported, and that no statistical analyses of FOB data were performed. These deficiencies did not change the conclusions of this review.

Reference

Goldey ES, Kehn LS, Rehnberg GL, Crofton KM. Effects of Developmental Hypothyroidism on Auditory and Motor Function in the Rat. Tox Appl Pharmacol 1995, 135: 67-76.

# DATA FOR ENTRY INTO ISIS

Developmental Neurotoxicity Study - rats (870.6300)

| PC code | MRID # | Study type | Species | Duration | Route | Dosing method | Dose range mg/kg/day | Doses tested mg/kg/day | NOAEL mg/kg/day | LOAEL mg/kg/day | Target organ(s) | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 128008 | 45404907 | dev neurotox | rats | GD 6-LD21 | oral | diet | 100-10000 | 100, 1000, 10000 | 1442 mg/kg/day | Not observed | | Maternal, dose in ppm |
| 128008 | 45404907 | dev neurotox | rats | GD 6-LD21 | oral | diet | 100-10000 | 100, 1000, 10000 | Not observed | 14 mg/kg/day | CNS; acoustic startle reflex amplitude | Offspring, dose in ppm |
| | | | | | | | | | | | | |



47