# DATA EVALUATION RECORD

## BAS 510 F

## STUDY TYPE: SUBCHRONIC NEUROTOXICITY – RAT (OPPTS 870.6200b/OECD 424) MRID 45404825

Prepared for

7/23/2002

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 02-06

| | | |
|---|---|---|
| Primary Reviewer: Rosmarie A. Faust, Ph.D. | Signature: | Robert H. Ross for R.A. Faust |
| | Date: | JAN 30 2002 |
| Secondary Reviewers: Sylvia S. Talmage, Ph.D., D.A.B.T. | Signature: | Sylvia S. Talmage |
| | Date: | JAN 30 2002 |
| Robert H. Ross, M.S., Group Leader | Signature: | Robert H. Ross |
| | Date: | JAN 30 2002 |
| Quality Assurance: Lee Ann Wilson, M.A. | Signature: | L. A. Wilson |
| | Date: | JAN 30 2002 |

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725.

(1)

**EPA Reviewer:** Alan Levy, Ph.D.
**Registration Action Branch 2, Health Effects Division (7509C)**
**EPA Secondary Reviewer:** Ghazi Dannan, Ph.D.
**Registration Action Branch 3, Health Effects Division (7509C)**

**Signature:** Alan C. Levy
**Date** 7-23-2002
**Signature:** _____
**Date** _____

Template version 11/01

**DATA EVALUATION RECORD**
**TXR#:** 0050193

**STUDY TYPE:** Subchronic Neurotoxicity - Rats OPPTS 870.6200b [§82-7], feeding - rats; OECD 424.

**PC CODE:** 128008

**DP BARCODE:** D278384
**SUBMISSION NO.:** S604279

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

**SYNONYMS:** 2-chloro-N-(4'-chloro-biphenyl-2-yl)-nicotinamide

**CITATION:** Mellert, W., W. Kaufmann, and B. van Ravenzwaay (2001) BAS 510 F - Subchronic oral neurotoxicity study in Wistar rats. Administration in the diet for 3 months. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, Germany. Report Project Number 50C0179/97148, BASF Registration Document Number 2001/1000113, February 6, 2001. MRID 45404825. Unpublished.

**SPONSOR:** BASF Corporation, Agricultural Products Division, Research Triangle Park, NC

**EXECUTIVE SUMMARY:** In a subchronic neurotoxicity study (MRID 45404825), groups of 10 male and 10 female 49-day old Chbb.THOM (SPF) Wistar rats were administered BAS 510 F (96.3% a.i., batch # N 46) in the diet at dose levels of 0, 150, 1500, or 15,000 ppm (equivalent to 0, 10.5, 103.1, or 1050.0 mg/kg bw/day for males and 0, 12.7, 124.5, or 1272.5 mg/kg/day for females) for 3 months. Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed in 10 animals/sex/group on days -7, 22, 50, and 85. Cholinesterase activities were not measured. At study termination, 5 animals/sex in the control and high-dose groups were subjected to *in situ* perfusion and neuropathological examination of brain and nervous system tissues.

No deaths occurred and there were no treatment-related clinical signs or effects on body weight and food consumption. FOB and motor activity testing revealed no treatment-related effects. Brain weights were comparable to controls and there were no gross or histopathologic findings that could be attributed to treatment with BAS 510 F.

**A LOAEL was not attained in this study. The NOAEL for BAS 510 F is 15,000 ppm in the diet (1050.0 mg/kg in male rats and 1272.5 mg/kg in female rats) based on the absence of**

2

**treatment-related systemic effects; absence of effects in the FOB and motor activity tests; and the absence of histopathological lesions in the brain and nervous system tissues.**

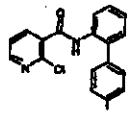
The study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for a subchronic neurotoxicity study in rats (870.6200b; OECD 424).

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

## I. MATERIALS AND METHODS:

### A. MATERIALS:

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

| | |
|---|---|
| **Description:** | white solid |
| **Batch #:** | N 46 |
| **Purity:** | 96.3% a.i. |
| **Compound Stability:** | expected to be stable for at least 2 years at room temperature or cooler |
| **CAS # of TGAI:** | 188425-85-6 |
| **Structure:** |  |

2. **Vehicle and/or positive control:** The test substance was administered in the food; no additional vehicle was used. The control diet was prepared in the same manner without the inclusion of the test substance.

3. **Test animals:**

| | | |
|---|---|---|
| **Species:** | Rat | |
| **Strain:** | Wistar [Chbb: THOM SPF] | |
| **Age/weight at study initiation:** | 49 days old: *males:* 232-271 g (mean 248 g); females: 159-194 g (177g) | |
| **Source:** | Boehringer Ingelheim Pharma KG | |
| **Housing:** | Singly, in type DK III stainless steel wire cages (Becker & Co., Castrop-Rauxel, Germany) | |
| **Diet:** | Kliba rats/mice/hamsters maintenance diet, meal (Provimi Kliba AG, Kaiseraugst, Switzerland), *ad libitum* | |
| **Water:** | Drinking water from water bottles, *ad libitum* | |
| **Environmental conditions:** | **Temperature:** | 20-24°C |
| | **Humidity:** | 30-70% |
| | **Air changes:** | Not given; room was air conditioned |
| | **Photoperiod:** | 12 hrs dark/12 hrs light |
| **Acclimation period:** | 13-16 days | |

### B. STUDY DESIGN:

1. **In life dates**: Start: February 22, 1999; end: May 28, 1999.

3

2. **Animal assignment and treatment:** Animals were assigned to test groups noted in Table 1 by means of a computerized randomization, stratified by body weight. In order to balance the groups for neurobehavioral testing (FOB and motor activity), dosing was staggered over 4 consecutive days. The study was conducted with 4 subsets, each subset consisting of either males (2 subsets, 5 males from each dose group) or females (2 subsets, 5 females from each dose group). All animals were 49 days old on the first day of test substance administration (i.e., day 0 for each subset). The staggered dosing schedule ensured that all animals were examined on the same day after start of dosing and on the same time of day, and all animals from all test groups could be used. The test substance was administered daily in the diet for 91 days.

   The dose selection rationale was based on the limit dose for a subchronic neurotoxicity study, 1000 mg/kg, according to guidelines. The high dose (15,000 ppm) provided a test substance intake of at least 1000 mg/kg body weight for males and females. The mid and low doses selected were 1500 ppm and 150 ppm, respectively. The mean daily test substance intake was equivalent to 0, 10.5, 103.1, or 1050.0 mg/kg bw/day (males) and 0, 12.7, 124.5, or 1272.5 mg/kg/day (females) for dietary concentrations of 0, 150, 1500, or 15,000 ppm.

**TABLE 1. Study design**

| Experimental Parameter | Dose Group (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 1500 | 15,000 |
| Doses (mg/kg/day) | | | | |
| Males | 0 | 10.5 | 103.1 | 1050.0 |
| Females | 0 | 12.7 | 124.5 | 1272.5 |
| Total number of animals/sex/group | 10/sex | 10/sex | 10/sex | 10/sex |
| Behavioral testing (FOB, Motor Activity) | 10/sex | 10/sex | 10/sex | 10/sex |
| Neuropathology* | 5/sex | 5/sex | 5/sex | 5/sex |

Data taken from pp. 17-18, 21, and 35, MRID 45404825.
*Tissues of animals in the 150 and 1500 ppm groups not examined microscopically.

3. **Test Substance preparation and analysis:** The test diet was prepared at intervals for which the stability of the test substance was guaranteed (32 days at room temperature). Appropriate amounts of test substance were mixed in a laboratory mixer with appropriate amounts of ground Kliba rats/mice/hamsters maintenance diet and stored at room temperature. The stability of the test substance in the diet was determined before the start of the study over a period of up to 32 days at room temperature. Homogeneity of the test substance preparation was analyzed at the start of the dosing period from three samples each taken from the highest and lowest dose levels. These samples also were used for concentration analyses. Additionally, one mid-dose sample was analyzed at the beginning of dosing and samples for all dose levels were analyzed at the end of the dosing period. The analytical method employed was HPLC with UV detection.

   **Results** -
   **Homogeneity analysis:** Analytical measurements of three samples each taken from the highest and lowest dose levels showed that actual concentrations (±SD) were 110.8±1.1% or 94.0±1.6% of the target concentration for test diets containing 150 or 15,000 ppm, respectively.

4

**Stability analysis:** The stability of the test substance in rodent feed was determined in three samples each (nominal content 100 mg/kg feed) taken on day 0, day 13, and day 32 at room temperature. The mean concentrations were 97.5% (day 13) and 97.8% (day 32) of the initial value (day 0).

**Concentration analysis:** Analysis of three samples taken before dosing varied between 109.9 and 112.1% of the target concentration at 150 ppm (mean 110.8%±1.1) and between 92.7 and 95.8% at 15,000 ppm (mean 94.0%±1.6). One 1500-ppm sample was 92.5% of the target concentration. The concentration of samples taken at the end of dosing were 100.0%, 94.5%, and 101.5% of the target concentration at 150, 1500, and 15,000 ppm, respectively.

The analytical data indicated that the mixing procedure was adequate and that stability and concentration are appropriate for the purpose of this study.

4. **Statistics:** Quantitative continuous data, e.g., body weight, body weight change, food consumption, and food efficiency, were analyzed by parametric one-way analysis using the F-test (ANOVA, two-sided). If the resulting p-value was ≤ 0.05, a comparison of each dose group with the control group was conducted using the Dunnett's test (two-sided) for the hypothesis of equal means.

   For feces, rearing, grip strength of forelimbs and hindlimbs, landing foot splay, motor activity, and brain weight, nonparametric one-way analysis of variance using the Kruskall-Wallis test (two-sided) was used. If the p-value was ≤ 0.05, a pairwise comparison of each dose group with the control group was carried out using the Mann-Whitney-U-test (two-sided) or Wilcoxon test (brain weight only) for equal medians. The reviewer considers the statistical analyses conducted for this study appropriate.

## C. METHODS/OBSERVATIONS:

1. **Mortality and clinical observations:** Animals were observed twice daily during the week and once daily on weekends and holidays for signs of toxicity and mortality. Detailed clinical observations and palpations were performed once a week.

2. **Body weight:** Animals were weighed before the first neurobehavioral test in order to randomize the animals. During the study, the animals were weighed on day 0 (start of dosing), and weekly thereafter. In addition, body weights were determined on the days when FOBs were conducted (days -7, 22, 50, and 85). Body weight change was calculated by determining the difference in body weight on the respective day of weighing and body weight on day 0.

3. **Food consumption and food efficiency:** Food consumption was recorded weekly and calculated as food consumption in g/animal/day. Food efficiency (group means ) was calculated by the study authors as [(g body weight gain/day)/(g food consumed/unit time)] x 100. The mean daily test substance intake (in mg/kg/day) was calculated for each dose group by sex based on individual body weight values and food consumption.

4. **Neurobehavioral assessment:**

a. **Functional Observational Battery (FOB):** All animals (10/sex/group) were subjected to a baseline FOB once before dosing (day -7) and on days 22, 50, and 85, each time starting at about 10 a.m. The FOB consisted of home cage observations, open field observations, and sensorimotor/reflex tests. Home cage observations were performed in closed cages and any disturbing activities were avoided. For open field observations, the animals were transferred to a standard arena (50 x 50 cm with 25 cm-high sides) and observed for at least 2 minutes. After removal from the open field arena, the animals were subjected to sensorimotor and reflex tests. Grip strength was measured using the procedure by Meyer et al. (Neurobehavioral Toxicology 1: 233-236, 1979). Where appropriate, the tested parameters were ranked for severity.

The examinations were carried out by trained technicians who had also performed positive control studies as part of their training. The technicians were blind to the treatment of the animals and the animals were tested in randomized order. The cages were randomly distributed in the racks at least 30 minutes prior to examination. The findings were recorded by a technician able to identify the animals.

The CHECKED (X) parameters were examined.[a]

| X | HOME CAGE OBSERVATIONS | X | HANDLING OBSERVATIONS | X | OPEN FIELD OBSERVATIONS |
|---|---|---|---|---|---|
| X | Posture* | X | Reactivity* | - | Mobility |
| - | Biting | X | Lacrimation* / chromodacryorrhea | X | Rearing+ |
| X | Convulsions* | X | Salivation* | X | Arousal/ general activity level* |
| X | Tremors* | X | Piloerection* | X | Convulsions* |
| X | Abnormal Movements* | X | Fur appearance | X | Tremors* |
| - | Palpebral closure* | X | Palpebral closure* | X | Abnormal movements* |
| - | Feces consistency | X | Respiratory rate+ | X | Urination / defecation* |
| | | - | Red/crusty deposits* | X | Gait abnormalities / posture* |
| | SENSORY OBSERVATIONS | X | Skin appearance | X | Gait score* |
| X | Approach response+ | X | Eye prominence* | X | Bizarre/stereotypic behavior* |
| X | Touch response+ | - | Muscle tone* | - | Backing |
| X | Startle response* | | | - | Time to first step |
| X | Pain response* | | | | |
| X | Pupil response* | | | | |
| X | Eyeblink response | | PHYSIOLOGICAL OBSERVATIONS | | NEUROMUSCULAR OBSERVATIONS |
| - | Forelimb extension | - | Body weight* | - | Hindlimb extensor strength |
| - | Hindlimb extension | - | Body temperature+ | X | Forelimb grip strength* |
| X | Air righting reflex+ | | | X | Hindlimb grip strength* |
| X | Olfactory orientation | | | X | Landing foot splay* |
| X | Vocalization | | OTHER OBSERVATIONS | - | Rotarod performance |
| X | Pinna reflex | | | | |

*Required parameters; +Recommended parameters - Not specifically mentioned as observed/recorded
[a]Some parameters were observed under other headings.

b. **Locomotor activity:** The animals were tested following each FOB session, from 2 p.m. onward. Motor activity was measured in the dark using the Multi-Varimex-System (Columbus Instruments Int. Corp., Ohio) with 4 infrared beams/cage. The number of beam interruptions were counted over 12 intervals, each lasting 5 minutes. The animals were put in randomized order into polycarbonate cages with wire covers (floor area about 800 $cm^2$ ) and did not receive food or water during the test session. Testing was staggered, starting for each animal when the first beam was interrupted and ending 60 minutes later. Data were presented for total motor activity over the 60-minute session and activity for each of the 5-minute intervals.

5. **Sacrifice and pathology:** At the end of the dosing period, five animals/sex/dose were selected for neuropathological evaluation of organs and tissues. After a fasting period of 16-20 hours, the animals were anesthetized with Narcoren® (4 ml/kg body weight) and then sacrificed by perfusion fixation. The perfused animals were necropsied and visible organs were examined grossly. Collected tissues were rinsed with Soerensen's phosphate buffer and fixed in Karnovsky solution. The remaining animals were sacrificed under $CO_2$ anesthesia without further examination.

Tissues were saved from all perfused groups; however, only tissues from control and high-dose animals were processed for histopathology and examination by light microscopy. Peripheral nervous system tissues (dorsal root fibers and ganglia, ventral root fibers and sections of sciatic, tibial, and sural nerve) were embedded in plastic (epoxy resin), followed by semithin

7

sectioning and staining with Azure II-methylene blue basic Fuchsin (Ambf). Central nervous system tissues (brain and spinal cord cross sections) as well as Gasserian ganglia with nerve and gastrocnemius muscle sections were embedded in paraffin, sectioned, and stained with hematoxylin-eosin.

Brain weights (without olfactory bulbs) of the 5 perfused animals were determined after removal from the body but before further preparation.

Additionally, the following tissues/organs were preserved in 4% formaldehyde: brain (remaining material after trimming), spinal cord (parts of cervical and lumbar cord), one hindlimb, and all gross lesions.

The CHECKED (X) tissues were evaluated.

| | CENTRAL NERVOUS SYSTEM | | PERIPHERAL NERVOUS SYSTEM |
|---|---|---|---|
| | **BRAIN** | | **SCIATIC NERVE** |
| X | Forebrain | X | Proximal sciatic nerve |
| X | Parietal lobe with diencephalon | | **OTHER** |
| X | Midbrain | X | Sural Nerve (at knee) |
| X | Cerebellum | X | Tibial Nerve (at knee) |
| X | Pons | X | Lumbar dorsal root ganglion |
| X | Medulla oblongata | X | Lumbar dorsal root fibers |
| | **SPINAL CORD** | X | Lumbar ventral root fibers |
| X | Cervical swelling | X | Cervical dorsal root ganglion |
| X | Lumbar swelling | X | Cervical dorsal root fibers |
| | **OTHER** | X | Cervical ventral root fibers |
| X | Gasserian Ganglion | | |
| X | Gastrocnemius muscle | | |
| | | | |

6. **Positive controls:** Positive control data providing evidence of the observational methods to detect major neurotoxic endpoints, increases and decreases in motor activity, and central and peripheral neuropathology were provided in summary form in Vol III (Supplement). Positive control chemicals included acrylamide, trimethyltin chloride, 3,3'-iminopropionitrile, carbaryl, nomifensin, and diazepam. The summaries were dated March 1, 1999; dates for the studies were not provided.

In the study with acrylamide (BASF Project No. 99C0259/89112), groups of 10 Wistar rats/sex were administered acrylamide by gavage as aqueous solutions at doses of 0 or 40 mg/kg bw for two weeks (5 doses/week, with a total of 11 doses). The study provided evidence to detect splay of the toes of the hindlimbs and ataxia; reduced activity and retarded tail pinch reaction; decreased grip strength of fore- and hindlimbs; and increased landing foot splay values. Treatment-related neuropathological lesions were seen in the cerebral cortex, spinal ganglion, peripheral nerves, and some nerve fibers of the gastrocnemius muscle (figures of the stained sections of the cerebral cortex, tibial nerve, and gastrocnemius muscle were provided).

Trimethyltin chloride (BASF Project No. 99S0228/93025) was administered intraperitoneally to groups of 5 male Wistar rats at single doses of 0, 6, 9, or 12 mg/kg bw. Clinical signs and

8

FOB observations included ataxia, tremors, convulsions, reduced grip strength, increased foot splay values, and increased motor activity. It was stated that the study demonstrated interobserver reliability for all technicians performing the FOBs. All test substance-related findings were detected by all observers to the same degree, and quantitative parameters of the controls were comparable. However, data for different technicians were not provided. Neuropathological findings included lesions in the olfactory bulb, brain (frontal and parietal lobes, midbrain, pons with cerebellar cortex, midcerebellum and medulla oblongata), cervical and lumbar spinal cord and ganglia, and peripheral nerves. Figures of the stained sections of dentate gyrus of the hippocampus region, Ammon's horn, cervical cord, and lumbar ganglion were provided.

In the study with 3,3'-iminodipropionitrile (BASF Project No. 99S0120/89004), Wistar rats (5/sex) were given a single intraperitoneal injection of 2 g/kg/bw. The rats exhibited abnormal clinical signs such as salivation, ataxia, walking backwards, circling movements, head twitching, lack of pupil reaction, no reaction to auditory stimulus, and reduced grip strength. Microscopically, peripheral nervous system and optic nerve lesions were observed.

Several studies were conducted with carbaryl (BASF Project Nos. 99C0378/94047, 99C0378/94052, and 99C0378/94077). The chemical was administered as a single intraperitoneal injection to Wistar rats at dose levels of 0, 10, or 30 mg/kg bw. Treatment-related and dose-dependent clinical signs included salivation, lacrimation, tremors, ataxia, and/or squatting posture. It was stated that the studies with carbaryl were conducted about once a year and demonstrated the interobserver reliability of the technicians performing FOBs. Additional details were not provided.

Nomifensin and diazepam (BASF Project No. 99C0378/94068) were studied to show that these chemicals increase or decrease motor activity, respectively. Nomifensin was administered as a single oral dose of 10 mg/kg bw to male and female Wistar rats. During the entire measurement period, motor activity was increased in both sexes. Diazepam was administered as a single oral dose of 3 mg/kg bw. Both sexes exhibited decreased motor activity, with habituation occurring much earlier than in controls.

Historical control data were provided for grip strength of fore- and hindlimbs, landing foot splay, and motor activity from 26 different neurotoxicity studies conducted at BASF (Tables 13-28, Vol. III Supplement). Although concurrent control data were used in the evaluation of the present study, historical control data provided information on normal ranges for untreated animals.

The positive control data were acceptable as demonstrations of the ability to detect various neurotoxic endpoints.

## II. RESULTS

### A. OBSERVATIONS:

1. **Clinical signs:** No clinical signs related to test substance administration were observed in any of the dose groups. One female administered 150 ppm injured the left forelimb during days 84-91 of the study.

2. **Mortality:** No deaths occurred in any of the dose groups.

**B. BODY WEIGHT AND BODY WEIGHT GAIN:** Mean body weights and body weight gains for selected weeks are listed in Table 2. No effects on body weight and body weight gain considered treatment-related were observed in any of the dose groups. At termination of the study, mean body weights in the dosed groups ranged from 97.6% to 103.8% of the control values for males and from 97.4% to 103.9% of the control values for females. The corresponding final weight gains ranged from 95% to 111% of controls (males) and from 91% to 133% of controls (females). Beginning approximately at day 70 (not shown in Table 2), slightly (n.s.) lower body weights and body weight gains were seen in high-dose female rats.

**TABLE 2. Body weight and body weight gain (g ± s.d.)**

| Observation | Dose Level (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 1500 | 15000 |
| **Body weight-Males** | | | | |
| Day 0 | 247.8±7.3 | 248.4±11.4 | 249.0±11.4 | 248.7±10.9 |
| Day 7 | 292.5±10.6 | 295.7±17.0 | 298.7±16.2 | 297.8±15.1 |
| Day 35 | 395.6±20.7 | 392.3±25.7 | 404.8±27.6 | 398.9±23.8 |
| Day 91 | 493.3±26.5 | 481.7±43.2 (97.6) | 512.3±38.8 (103.8) | 501.4±38.1 (101.6) |
| **Body weight gain-Males** | | | | |
| Day 7 | 44.7 | 47.3 (106) | 49.7 (111) | 49.1 (110) |
| Day 35 | 147.8 | 143.9 (97) | 155.8 (105) | 150.2 (102) |
| Day 91 | 245.5 | 233.3 (95) | 263.3 (107) | 252.7 (103) |
| | Dose Level (ppm) | | | |
| | 0 | 150 | 1500 | 15000 |
| **Body weight-Females** | | | | |
| Day 0 | 176.0±8.8 | 177.1±10.2 | 178.1±9.1 | 177.4±7.6 |
| Day 7 | 189.6±10.3 | 189.5±13.8 | 196.2±9.2 | 190.3±5.8 |
| Day 35 | 232.4±14.5 | 235.1±22.4 | 244.7±9.3 | 234.2±10.6 |
| Day 91 | 281.9±18.0 | 283.7±26.2 (100.6) | 293.0±9.5 (103.9) | 274.5±17.3 (97.4) |
| **Body weight gain-Females** | | | | |
| Day 7 | 13.6 | 12.4 (91) | 18.1 (133) | 12.9 (95) |
| Day 35 | 56.4 | 58.0 (103) | 66.6 (118) | 56.8 (101) |
| Day 91 | 105.9 | 106.6 (101) | 114.9 (108) | 97.1 (92) |

Data were extracted from MRID 45404825, Tables 1A7-1A14, pp. 64-71.
Values represent mean ± s.d.
n=10 animals/sex/group
Values in parenthesis represent percent of control values.

C. **FOOD CONSUMPTION and FOOD EFFICIENCY:** No treatment-related effects on food consumption were observed in any of the dose groups. In general, food efficiency steadily decreased in all test groups and both sexes, including controls, over the course of the 3-month study period. Males administered 150 ppm had a significantly ($p \leq 0.05$) decreased food efficiency on day 63 only. This deviation was considered incidental and not biologically significant.

D. **CHOLINESTERASE ACTIVITIES**: Data on cholinesterase activities were not provided.

E. **NEUROBEHAVIORAL RESULTS:**

1. **FOB Findings:** In the FOB, no statistically significant findings were recorded in any of the dose groups at any time point. The following findings were considered clearly incidental in nature: On day 50, one low-dose male exhibited very frequent vocalizations when touched and, on day 85, one control female slightly resisted handling upon removal from cage. In addition, the study author's summary of FOB results (Vol. I, p. 38) reported piloerection in two high-dose females during open field observations on day 22. However, this effect was not recorded in group summary tables (Table IA-51, p. 108) nor in tables of individual animal values (Table IIA-159, p. 300) and, therefore, appears questionable.

   Treatment with BAS 510 F had no effect on hindlimb or forelimb grip strength or on foot splay for either sex. Rearing activity in the open field was highly variable in all groups and both sexes, ranging from 45.5% to 140.9% of controls for males and from 75.6% to 128.2% of controls for females, but showed no statistically significant deviations. No assessment was possible for a few parameters. These included impairment of gait during home cage observations (animals did not walk during the observation period) and fecal and urinary output during open field observations (no defecation or urination during the observation period).

2. **Motor activity:** Total motor activity counts for the 60-minute session, measured as beam interruptions, are summarized in Table 3. No statistically significant increases or decreases in total motor activity were recorded. Motor activity in dosed males ranged from 120.9% (day 22, 15,000 ppm) to 77.6% of the control value (day 85, 15,000). Data for females were also variable, ranging from 138.2% (day 85, 15,000 ppm) to 98% (day 50, 1500 ppm) of controls. Pretreatment (day -7), motor activity ranged from 97.1% to 124.6% of controls for males and from 107.5% to 131.8% (1500 ppm, $p \leq 0.05$) of controls for females. These results reflect the inherent variability of this type of test. Dose-related trends were observed in male and female rats on day 22 (increased activity) and in male rats on day 85 (decreased activity). Also noted were trends across time in the high-dose groups (males, decreased activity; females, increased activity).

   Data from the subsessions showed a decline in activity with succeeding intervals for all groups at each test week. A dramatic decline in motor activity occurred between subsessions 6 and 8, indicating similar habituation. The lowest activities were generally observed between subsessions 8 and 12. During the 60-minute session, there were isolated incidences of increases or decreases in motor activity.

The study authors considered the above noted deviations in total motor activity and motor activity during the single intervals incidental to treatment due to the isolated occurrence and lack of a dose-response relationship.

**TABLE 3. Motor activity (total activity counts for session)**

| Test Day | Dose Level (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 1500 | 15000 |
| **Males** | | | | |
| Day -7 | 103±35 | 100±30 (97.1) | 128±47 (124.6) | 126±38 (122.6) |
| Day 22 | 159±45 | 145±36 (91.1) | 171±81 (107.4) | 192±66 (120.9) |
| Day 50 | 156±48 | 172±44 (110.1) | 182±62 (116.4) | 175±47 (111.9) |
| Day 85 | 161±48 | 164±32 (101.7) | 158±46 (98.1) | 125±26 (77.6) |
| | Dose Level (ppm) | | | |
| | 0 | 150 | 1500 | 15000 |
| **Females** | | | | |
| Day -7 | 137±43 | 159±27 (115.6) | 181±39* (131.8) | 147±22 (107.5) |
| Day 22 | 197±66 | 222±49 (112.7) | 244±93 (124.0) | 252±48 (128.1) |
| Day 50 | 189±54 | 194±41 (103.0) | 185±83 (98.0) | 242±53 (128.4) |
| Day 85 | 168±49 | 216±76 (129.1) | 195±54 (116.4) | 232±44 (138.2) |

Data were extracted from MRID 45404825, Table IA, pp. 59-60, 116-117.
Values represent mean ± s.d.
n=10 animals/sex/group
Values in parenthesis represent percent of control values.
*$p \leq 0.05$, compared with controls

## F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** No gross lesions were recorded in any of the dose groups.

2. **Brain weight:** No statistically significant effects on absolute brain weights were recorded.

3. **Neuropathology:** The only lesion observed was a single axonal degeneration in the dorsal root ganglia of the lumbar region in one male and one female administered the high dose. The lesion was classified as grade 1 (minimal severity.) The study authors considered this lesion spontaneous in nature and not related to treatment. The lower dose animals were not examined.

## III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The study authors concluded that there were no treatment-related clinical signs of toxicity or adverse effects on body weight and food consumption. There was no mortality. No signs of neurotoxicity were observed during FOBs, motor activity measurements, and neuropathologic examinations. Therefore, based on general toxicity and neurotoxicity, the NOAEL was the highest dietary concentration tested, 15,000 ppm (1,050.0 mg/kg bw in males; 1,272.5 mg/kg bw in females) under the conditions of this study.

B. REVIEWER COMMENTS: Male and female Chbb.THOM (SPF) Wistar rats were administered BAS 510 F in the diet for 3 months at concentrations of 0, 150, 1500, or 15,000 ppm. Based on analytical measurements, the actual doses were 0, 10.5, 103.1, or 1050.0 mg/kg/day for males and 0, 12.7, 124.5, or 1272.5 mg/kg/day for females. The selection of dose levels appeared to be appropriate.

At study termination, slightly (n.s.) decreased body weights and body weight gains (<10%) were observed in female rats at 15,000 ppm. The effect on body weight and body weight gains may be treatment-related; however, the reviewer considers the low magnitude of the weight decrease not toxicologically significant. Food consumption and food efficiency were not significantly affected.

No effects clearly attributable to the test substance indicating neurotoxicity were observed at any dose level. Scattered findings in the FOB and motor activity evaluations are of questionable biological significance. In the FOB, piloerection during open field observations (reported only in the study summary) was observed in two females in the 15,000 ppm group after 3 weeks of test substance administration. This effect was not present at any other observation time or in males in any dose group at any observation time. [It should be noted that piloerection was observed on the day of dosing in 2/10 female Wistar rats administered a single oral dose of 2000 mg/kg bw of BAS 510 F in the acute neurotoxicity study (MRID 45404820)].

Increased total motor activity greater than 20% (suggested acceptable limit) was recorded in mid-dose females on day 22 ($p \leq 0.05$, 124% of controls) and in high-dose females on days 22, 50, and 85 (128.1%, 128.4%, and 138.2% of controls, respectively). The increases in the high-dose animals were not statistically significant and are considered moderate deviations. Motor activity counts by interval showed considerable variability among individual animals. Increases of motor activity by time interval were isolated and statistically significant ($p \leq 0.01$) in high-dose females during subsession 10 (day 50) and subsession 3 (day 85). In contrast to female rats, high-dose male rats exhibited decreased total motor activity (77.8% of controls) on day 85, corresponding to decreased ($p \leq 0.05$) activity counts during subsession 10 on day 85. The motor activity changes were not accompanied by clinical signs suggesting increased or decreased activity/alertness levels. In light of these observations, it is difficult to consider motor activity as a neurotoxic effect of the test material.

There were no gross lesions of the nervous system or effects on brain weight. Histopathological examination of central and peripheral nervous system tissues revealed slight axonal degeneration in the lumbar dorsal root ganglia in one male and one female administered 15,000 ppm. This effect is not considered treatment-related due to the minimal severity and lack of correlation with functional effects in those animals. It should be noted that data on the microscopic lesions of the nervous system were presented only in summary tables without details. However, data presented for positive controls indicated that the investigators are capable of observing treatment-related lesions.

The reviewer agrees with the Investigators' conclusions concerning the NOAEL of the study.

**The NOAELs for BAS 510 F in male and female rats are 1050.0 mg/kg bw and 1272.5 mg/kg bw, respectively. LOAELs were not attained in either sex.**

13

C. **STUDY DEFICIENCIES:** There were no major study deficiencies. The batch number of the material tested for stability was listed as N 21, whereas the batch number of the test material used in the study and tested for concentration and homogeneity was listed as N 46. Some of the FOB summary tables were difficult to interpret, such as the inability to observe gait in the home cage because the animals were not moving, and absence of fecal and urinary output during open field observations. Positive control studies appear to have been properly conducted; however some details were not provided, e.g., number of technicians involved to demonstrate interobserver reliability and study dates. These shortcomings do not invalidate the study.

# DATA FOR ENTRY INTO ISIS

Subchronic Neurotoxicity Study - rats (870.6200b)

| 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 | 45404825 | subchronic neurotoxicity | rats | 90 days | oral | diet | males: 10.5-1050.0; females: 12.7-1272.5 | males: 0, 10.5, 103.1, 1050.0; females: 0, 12.7, 124.5, 1272.5 | males: 1050.0; females: 1272.5 | not attained | None | |



51