Secondary Reviewer: Meta Bonner, Ph.D.
US EPA, OPPT, OPP, HED, RAB3, 7905c

Date: July 18, 2001

**US EPA Assessment of PMRA Data Evaluation Record**

**Study Type:** Acute Neurotoxicity - Rats OPPTS 870.6100

**DP Barcode:** D269669, D267732
**PC Code:** 099100

**Submission Code:** S583112
**Tox. Chem. No.:** none

**Test Material (Purity):** BAS 500 F; purity 99.0%

**Synonyms:** Reg. No. 304 423, Pyraclostrobin

**Citation:** Mellert, W., Kaufmann, W., and Hildebrand, B. (1999) BAS 500 F - Acute Oral Neurotoxicity Study in Wistar Rats. Department of Toxicology of BASF Aktiengesellschaft, Rhein, FRG. Laboratory Project No. 20C0494/96164, BASF Doc. No. 1999/11111; August 18, 1999. MRID # 45118337.

**Sponsor:** BASF Canada Inc., Agricultural Products, Toronto, Ontario

**Executive Summary:** In an acute neurotoxicity study (MRID # 45118337), groups of 10 Chbb:THOM (SPF) Wistar rats/sex were given a single oral dose of BAS 500 F (purity 99.0%) in a 0.5% aqueous solution of carboxymethylcellulose at dose levels of 0, 100, 300, and 1000 mg/kg bw and observed for 14 days. Neurobehavioral assessments were performed on all animals at ~4 to 6 hours, 7 days and 14 days post-dosing. At study termination, 5 animals/sex/group were euthanized, perfused in situ and subjected to histopathological evaluation of central and peripheral nervous system tissues. The Systemic Toxicity LOAEL for males was 1000 mg/kg bw based on decreased body weight gain. The Systemic Toxicity NOAEL was 300 mg/kg bw. The Systemic Toxicity LOAEL for females could not be determined since there were no adverse, treatment-related effects noted at any dose level tested. The Systemic Toxicity NOAEL for females was 1000 mg/kg bw. The Neurotoxicity LOAEL could not be determined since there were no treatment-related neurotoxic effects noted at any dose level tested. The Neurotoxicity NOAEL was 1000 mg/kg bw.

This acute neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute neurotoxicity study [OPPTS 870.6100(§81-7)] in rats.

**EPA Reviewer Comments and Conclusions:** My evaluation of the acute neurotoxicity study concurs with the conclusions reached by the PMRA reviewer. However, the following few errors

were noted in the PMRA review: (1) OPPTS 870.6100 instead of 870.6200; (2) In life dates of June 1, 1998 to June 16, 1998 instead of June 9, 1998 to June 16, 1998.

Reviewer: Brenda MacDonald, D.V.M. , Date: April, 2001

**STUDY TYPE:** Acute Neurotoxicity - Rats OPPTS 870.6200; OECD 424.

**TEST MATERIAL (PURITY):** BAS 500 F; purity 99.0%

**SYNONYMS:** Reg. No. 304 428, Pyraclostrobin

**CITATION:** Mellert, W., et al (1999) BAS 500 F - Acute Oral Neurotoxicity Study in Wistar Rats. Department of Toxicology of BASF Aktiengesellschaft, Rhein, FRG. Laboratory Project No. 20C0494/96164, BASF Doc. No. 1999/11111, August 18, 1999. MRID #45118337. Unpublished.

**SPONSOR:** BASF Canada Inc., Agricultural Products, Toronto, Ontario

**EXECUTIVE SUMMARY:** In an acute neurotoxicity study (MRID #45118337), groups of 10 Chbb:THOM (SPF) Wistar rats/sex were given a single oral dose of BAS 500 F (purity 99.0%) in a 0.5% aqueous solution of carboxymethylcellulose at dose levels of 0, 100, 300 and 1000 mg/kg bw and observed for 14 days. Neurobehavioral assessments (functional observation battery and motor activity testing) were performed on all animals at ~4 to 6 hours, 7 days and 14 days post-dosing. At study termination, 5 animals/sex/group were euthanized, perfused *in situ* and subjected to histopathological evaluation of central and peripheral nervous system tissues. The remaining 5 animals/sex/group were sacrificed by $CO_2$ inhalation and discarded. All animals survived the 14-day observation period, and there were no treatment-related clinical signs of toxicity. Decreased body weight gain was noted for males only in the 1000 mg/kg bw group during the first week of the study. During the 2 minute open field observations an increased incidence of soft feces, mucoid feces and/or diarrhea was noted at all dose levels on day 0 only (day of test material administration). In addition, piloerection was observed on day 0 in the high dose group, females only. However, these findings were not considered to be toxicologically significant since they were not observed on day 0 or any other time during the 2 week clinical observation period. There were no other treatment-related findings noted during the FOBs at 0, 7 or 14 days. There was no treatment-related effect on motor activity at any dose level tested. Gross and histopathological examination of the central nervous system and the peripheral nervous system did not reveal any treatment-related findings. The noted treatment-related findings were considered to be due to acute systemic toxicity and/or local effects on the digestive tract.

**Based on the effects seen in this study, the LOAEL for systemic toxicity for males was 1000 mg/kg bw based on decreased body weight gain. The NOAEL was 300 mg/kg bw. The LOAEL for females could not be determined since there were no adverse, treatment-related effects noted at any dose level tested. The NOAEL was 1000 mg/kg bw.**

**The LOAEL for neurotoxicity could not be determined since there were no treatment-related neurotoxic effects noted at any dose level tested. The NOAEL was 1000 mg/kg bw.**

The study is classified as acceptable as an acute neurotoxicity study in rats (870.6200; OECD 424).

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

# I. MATERIALS AND METHODS

## A. MATERIALS:

1 **Test Material:** BAS 500 F

| | |
|---|---|
| **Description:** | Technical, solid, crystalline, yellowish |
| **Lot/Batch #:** | LJ.-No. 28632/147FS; CP 029053 |
| **Purity:** | 99.0 % a.i. |
| **CAS #:** | 175013-18-0 |
| **Stability:** | Proven by reanalysis after the in-life phase of the study (purity 99.1%; analytical report no. PCP04960). |

2. **Vehicle:** 0.5% aqueous solution of carboxymethylcellulose in aqua bidest

3 **Test Animals:**

| | | |
|---|---|---|
| **Species:** | Rat | |
| **Strain:** | Wistar; Chbb:THOM (SPF) | |
| **Age/weight at dosing:** | 49 days of age<br>Body weight: Males, 206.9 g to 265.3 g; Females, 145.4 g to 196.7 g. | |
| **Source:** | Bochringer Ingelheim Pharma KG. | |
| **Housing:** | Individually in type DK III stainless steel wire cages; Motor activity measurements were conducted in Polycarbonate cages with wire covers. | |
| **Diet:** | Not stated | |
| **Water:** | Not stated | |
| **Environmental conditions:** | **Temperature:** | 20 to 24°C |
| | **Humidity:** | 30 to 70 % |
| | **Air changes:** | Not stated |
| | **Photoperiod:** | 12 hrs dark/12 hrs light |
| **Acclimation period:** | ~ 2 weeks | |

## B. STUDY DESIGN:

1. **In Life Dates:** June 9, 1998 to June 16, 1998

2. **Animal Assignment and Treatment:** Animals were randomly assigned to the test groups noted in Table 1 using a computer-generated randomization list based upon body weight.

**Table 1. Study Design**

| Experimental Parameter | Dose, mg/kg bw | | | |
|---|---|---|---|---|
| | 0 | 100 | 300 | 1000 |
| Total number of Animals/sex/group | 10 | 10 | 10 | 10 |
| Behavioral Testing (FOB, Motor Activity) | 10/sex | 10/sex | 10/sex | 10/sex |
| Neuropathology | 5/sex | 5/sex | 5/sex | 5/sex |

Rats were given a single dose of BAS 500 F dissolved in a 0.5% aqueous solution of carboxymethylcellulose by oral gavage (using a 5 cc resp. 10 cc syringe) at a dosing volume of 20 mL/kg bw. It was not stated whether the animals were fasted prior to dosing. Control animals received vehicle alone. The dosage volume administered was based on the body weight of each individual animal determined on day 0. During dosing the suspensions were stirred with a magnetic stirrer.

Doses were selected based on the results of a peak finding study in which rats were administered a single oral dose of BAS 500 F dissolved in 0.5% aqueous solution of carboxymethylcellulose, at concentrations of 1000 and 2000 mg/kg bw, 3/sex/group. One male and one female in the 2000 mg/kg bw group died on study days 3 and 4, respectively. Diarrhea was observed for animals in the 1000 and 2000 mg/kg bw groups on the day of administration. In addition, animals in the 2000 mg/kg bw group were apathetic on the day of administration, and exhibited piloerection on days 0 to 6. Time of peak effect was determined to be 4 to 8 hours after dosing. Based on these results, dose levels chosen for the main study were 1000 mg/kg bw (as high dose with expected toxic effects), 300 and 100 mg/kg bw.

**3. Test Substance Preparation and Analysis:** The test substance was prepared for oral dosing by mixing the appropriate amount of test material and 0.5% carboxymethylcellulose (CMC) solution to yield the desired concentration and volume, using a magnetic stirrer. Fresh solutions were prepared on the day of administration. Stability of the test material in aqueous CMC solution over a 4-day period at room temperature was determined prior to study initiation at the nominal concentration of 50 mg/100 mL. Homogeneity of the test material in CMC solution was determined at the start of the dosing period, at nominal concentrations of 0.5 and 5.0 g/100 mL, from samples taken from the top, middle and bottom of the suspension. Actual test material concentration was determined from samples taken at the start of dosing at all dose levels.

**Results:** **Homogeneity Analysis:** Individual samples at concentrations of 0.5 and 5.0 g/mL ranged from 92.6% to 94.8% of the nominal concentration and 89.8% to 94.8% of the nominal concentration, respectively.

**Stability Analysis:** The actual concentrations of BAS 500 F, expressed as percentage of the initial concentration, were as follows:

| Dose (mg/mL) | |
|---|---|
| Storage Interval, hr | 50 |
| 0 | 100.0 |
| 4 | 99.1 |
| 24 | 99.8 |
| 48 | 101.1 |
| 72 | 105.1 |
| 96 | 98.7 |

**Concentration Analysis:** Individual samples at concentrations of 0.5, 1.0 and 5.0 g/mL ranged from 92.6% to 94.8% of the nominal concentration, 98.7% to 99.4%, and 89.8% to 94.8% of the nominal concentration, respectively.

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

4. **Statistics:** Statistical analyses performed were as follows:
i) Body weight: Parametric one-way analysis using the F-test (ANOVA). If the resulting p-value was $\leq 0.05$, a comparison of each group with the control group was performed using Dunnett's two-sided test.
ii) Feces, rearing, grip strength forelimbs and hindlimbs, landing foot-splay test, motor activity: Non-parametric one-way analysis using two-sided Kruskal-Wallis test. If the resulting p-value was $\leq 0.05$, a pairwise comparison of each dose group with the control group was performed using the two-sided Mann-Whitney U-test.

## C. METHODS / OBSERVATIONS:

1. **Mortality and Clinical Observations:** Animals were observed twice daily during the week and once a day on the weekends and holidays for signs of toxicity and mortality. Detailed physical examinations were performed daily.

2. **Body Weight:** Animals were weighed on the days when the functional observational batteries were performed, i.e., on days -7, 0, 7 and 14.

3. **Food Consumption:** Not measured.

4. **Neurobehavioral Assessment:**

**a. Functional Observational Battery (FOB):** A functional observational battery was performed on all animals at approximately the same time of day for each assessment, once before initiation of treatment (day -7) and on study days 0 (~ 4 to 6 hours after dosing), 7 and 14. The observation technician was blind regarding the treatment status of the animals. During the open field observations, animals were observed for at least 2 minutes. The CHECKED (✓) parameters were evaluated.

| | HOME CAGE OBSERVATIONS | | HANDLING OBSERVATIONS | | OPEN FIELD OBSERVATIONS |
|---|---|---|---|---|---|
| ✓ | Posture | ✓ | Reactivity | ✓ | Mobility |
| ✓ | Impairment of gait | ✓ | Lacrimation / chromodacryorrhea | ✓ | Rearing |
| ✓ | Convulsions | ✓ | Salivation | ✓ | Arousal/ gereral activity level |
| ✓ | Tremors | ✓ | Piloerection | ✓ | Convulsions |
| ✓ | Abnormal Movements | ✓ | Fur appearance | ✓ | Tremors |
| | Palpebral closure | ✓ | Palpebral closure | ✓ | Abnormal movements |
| ✓ | General observations (all other abnormal findings) | ✓ | Respiratory rate | ✓ | Urination / defecation |
| | | ✓ | Red/crusty deposits | ✓ | Grooming |
| | SENSORY OBSERVATIONS | ✓ | Mucous membranes /eye /skin colour | ✓ | Gait abnormalities / posture |
| ✓ | Approach response | ✓ | Eye prominence | | Gait score |
| ✓ | Touch response | | Muscle tone | ✓ | Bizarre / stereotypic behaviour |
| ✓ | Startle response | ✓ | Behaviour during handling | | Backing |

| | | | | | |
|---|---|---|---|---|---|
| ✓ | Pain response | ✓ | Skin | | Time to first step |
| ✓ | Pupil response | | | | |
| ✓ | Eyeblink response | | **PHYSIOLOGICAL OBSERVATIONS** | | **NEUROMUSCULAR OBSERVATIONS** |
| ✓ | Pinna reflex | ✓ | Body weight | | Hindlimb extensor strength |
| ✓ | Vocalization | | Body temperature | ✓ | Forelimb grip strength |
| ✓ | Air righting reflex | | | ✓ | Hindlimb grip strength |
| ✓ | Olfactory orientation | | | ✓ | Hindlimb foot splay |
| ✓ | Examination of catalepsy ("descending from box") | | **OTHER OBSERVATIONS** | | Rotarod performance |
| ✓ | Vision ("visual placing response") | | | | |

**b. Motor Activity:** Motor activity was evaluated on the same days that the FOBs were performed (i.e., study days -7, 0, 7 and 14) at approximately the same time of day for each assessment. Measurements were performed in the dark using the Multi-Varimex-System with 4 infrared beams per cage. The period of assessment for each animal commenced when the first beam was interrupted and ended exactly 60 minutes later. The number of beam interrupts were counted over twelve 5-minute intervals. Animals were food and water fasted during assessment.

5. **Sacrifice and Pathology:** At the end of the study, 5 rats/sex/group were sacrificed by $CO_2$ anesthesia and discarded. The remaining 5 rats/sex/group were anesthetized with Nembutal and sacrificed by perfusion fixation using fixation solution according to Karnovsky. The rinsing solution used was Soerensen's phosphate buffer. Each animal was necropsied.

The CHECKED (✓) tissues were examined.

| | | | |
|---|---|---|---|
| | **CENTRAL NERVOUS SYSTEM** | | **PERIPHERAL NERVOUS SYSTEM** |
| | **BRAIN** | | **SCIATIC NERVE** |
| ✓ | Frontal lobe | ✓ | Proximal sciatic nerve |
| ✓ | Parietal lobe with diencephalon | | |
| ✓ | Midbrain with occipital and temporal lobe | | |
| ✓ | Cerebellum | | |
| ✓ | Pons | | **OTHER** |
| ✓ | Medulla oblongata | ✓ | Sural Nerve |
| | **SPINAL CORD** | ✓ | Tibial Nerve |
| ✓ | Cervical swelling | | Peroneal Nerve |
| ✓ | Lumbar swelling | ✓ | Dorsal root ganglion (C3-C6) |
| | Thoracic swelling | ✓ | Dorsal root fiber (C3-C6) |
| | | ✓ | Ventral root fiber (C3-C6) |
| | **OTHER** | ✓ | Dorsal root ganglion (L1-L4) |
| ✓ | Gasserian ganglia with nerve | ✓ | Dorsal root fiber (L1-L4) |
| | Trigeminal nerves | ✓ | Ventral root fiber (L1-L4) |
| | Optic nerve | | |
| | Eyes | | |
| ✓ | Gastrocnemius muscle | | |

8

In the control and 1000 mg/kg bw groups, brain, spinal cord, Gasserian ganglia and gastrocnemius muscle were embedded in paraffin, sectioned and stained with hematoxylin-eosin, then examined microscopically. In the 100 and 300 mg/kg bw groups, these tissues were preserved in 4% formaldehyde and stored. In the control and 1000 mg/kg bw groups, dorsal root fibers, ventral root fibers, tibial nerve and sural nerve were embedded in epoxy resin, sectioned and stained with Azure II-methylene blue-basic Fuchsin, then examined microscopically. In the 100 and 300 mg/kg bw groups, these tissues were preserved in 4% buffer solution and stored.

6. **Positive Controls:** Summaries of positive control studies were submitted, which used Functional Observational Batteries, Motor Activity Measurements and Neuropathology to evaluate behavioural and neuropathological findings of substances known to elicit nervous system effects. A summary of the study results follows.

i) In a subacute study conducted using acrylamide (BASF Project No. 99C0259/89112), males and females exhibited splay of the toes of the hindlimbs, ataxia, decreased activity, retarded reaction to tail pinch, decreased fore- and hindlimb grip strength and increased landing food splay values. Histopathological findings were selective Purkinje cell necrosis and vacuolation of the molecular layer in the cerebellar cortex, cytoplasmic remodelling in spinal ganglion cells, Wallerian-like axonal degeneration in peripheral nerves, neurofilament accumulation in some nerve fibers of intramuscular nerves, neurofilament accumulation and decrease in or loss of synaptic vesicles and swelling of synaptic terminals in neuromuscular junctions.

ii) In an acute study conducted using trimethyltin chloride (BASF Project No. 99S0228/930225), treatment-related findings included ataxia, tremors, convulsions, reduced grip strength, increased landing foot splay values and increased motor activity values. Histopathological findings were neuronal necrosis in the olfactory bulb, hydrocephalus internus in the frontal and parietal lobe, neuronal necrosis in the midbrain with cortex cerebri, purkinje cell necrosis in the pons with cerebellar cortex, midcerebellum and medulla oblongata with cerebellar cortex, chromatolysis of alpha-motor neurons in the cervical and lumbar cord, axonal degeneration in the cervical ganglia and peripheral nerves and vacuolar degeneration in the lumbar ganglia. It was stated that the same results were seen by all technicians performing FOBs in the laboratory, demonstrating interobserver reliability.

iii) In an acute study conducted using 3,3'-iminodiproprionitrile (BASF Project No. 99S0120/89004), treatment-related findings were salivation, ataxia, walking backwards, circling movements, head twitching, lack of pupil reaction, no reaction to auditory stimulus and reduced grip strength. Histopathological examination revealed axonal atrophy in the distal segments of peripheral nerves, intraocular hemorrhages and degeneration with atrophy of the retina and optic nerve.

iv) In 3 acute studies conducted using carbaryl (BASF Project Nos. 99C0378/94047, 99C0378/94052 and 99C0378/94077), findings included salivation, lacrimation, tremors and ataxia and/or squatting posture. Interobserver reliability was demonstrated between these studies.

v) In an acute study conducted using nomifensin and diazepam (BASF Project No. 99C0378/94068), nomifensin elicited increased motor activity during the entire measurement, whereas diazepam resulted in decreased motor activity and earlier habituation.

Based on these results, it is concluded that the positive control data base is acceptable for use with the current study.

Historical control data were submitted for rearing, forelimb and hindlimb grip strength, landing foot

9

splay and motor activity measurement.

## II. RESULTS

1. **Mortality:** All animals survived the duration of the study period.

2. **Clinical Observations:** No treatment-related differences in clinical signs were observed in any of the test groups immediately following dosing or during the 2-week observation period.

3. **Body Weight and Body Weight Gain:** Refer to Table 2. The only finding considered to be treatment-related was decreased body weight gain for males in the 1000 mg/kg bw group during the first week of the study.

**Table 2. Body Weight and Body Weight Gain (g)**

| Observation | Dose, mg/kg bw | | | |
|---|---|---|---|---|
| | 0 | 100 | 300 | 1000 |
| **Body Weight-Males** | | | | |
| Day 0 | 234.6±14.8 | 238.1±14.7 | 237.1±14.7 | 241.5±13.0 |
| Day 7 | 276.7±17.7 | 277.1±17.5 | 274.0±16.9 | 269.5±14.6 |
| Day 14 | 311.9±21.0 | 310.8±22.6 | 308.7±18.8 | 313.7±14.7 |
| **Body Weight-Females** | | | | |
| Day 0 | 164.9±14.3 | 168.9±12.0 | 169.3±10.8 | 165.8±11.9 |
| Day 7 | 183.9±15.7 | 190.4±12.4 | 185.1±14.0 | 184.6±15.2 |
| Day 14 | 196.2±19.2 | 203.6±11.9 | 200.6±13.4 | 199.8±16.1 |
| **Body Weight Gain-Males** | | | | |
| Day 0-7 | 42.1±4.3 | 39.0±4.4 | 36.9±5.4 | 28.0±11.2** |
| Day 0-14 | 77.3±8.2 | 72.6±11.8 | 71.6±7.7 | 72.2±9.4 |
| **Body Weight Gain-Females** | | | | |
| Day 0-7 | 19.0±2.6 | 21.6±3.8 | 15.8±4.9 | 18.8±4.4 |
| Day 0-14 | 31.3±8.9 | 34.8±4.7 | 31.3±5.2 | 34.1±5.4 |

Data were extracted from pages 53 to 56 in the study report. Values represent mean ± s.d.; n=5. *p<.0.05, **p<.0.01, when compared to control means.

4. **Food Consumption:** Not measured.

5. **Neurobehavioral Results:**

**a. FOB Findings:** Refer to Table 3. Soft feces, mucoid feces and/or diarrhea were observed at all dose levels, both sexes, on the day of treatment (day 0) only. The only other treatment-related finding was piloerection observed in the 1000 mg/kg bw group, females only, on the day of treatment. However, none of these findings were considered to be toxicologically significant since they were only observed during the 2-minute open field observations, but not on day 0 or any other time during the 2 week clinical observation period.

**Table 3. Functional Observation Battery Results**

| Observation | Dose (mg/kg bw) | | | |
|---|---|---|---|---|
| | 0 | 100 | 300 | 1000 |
| **Males** | | | | |
| **Diarrhea** | | | | |
| Pretest | 0/6 (n=4) | 0/9 (n=1) | 0/7 (n=3) | 0/8 (n=2) |
| Day 0 | 0/8 (n=2) | 0/7 (n=3) | 2/8 (n=2) | 5/8 (n=2) |
| Day 7 | 0/8 (n=2) | 0/6 (n=4) | 0/7 (n=3) | 0/4 (n=6) |
| Day 14 | 0/4 (n=6) | 0/4 (n=6) | 0/4 (n=6) | 0/4 (n=6) |
| **Soft feces** | | | | |
| Pretest | 0/6 (n=4) | 0/9 (n=1) | 0/7 (n=3) | 0/8 (n=2) |
| Day 0 | 0/8 (n=2) | 3/7 (n=3) | 5/8 (n=2) | 1/8 (n=2) |
| Day 7 | 0/8 (n=2) | 0/6 (n=4) | 0/7 (n=3) | 0/4 (n=6) |
| Day 14 | 0/4 (n=6) | 0/4 (n=6) | 0/4 (n=6) | 0/4 (n=6) |
| **Females** | | | | |
| **Diarrhea** | | | | |
| Pretest | 0/2 (n=8) | 0/3 (n=7) | 0/3 (n=7) | 0/2 (n=8) |
| Day 0 | 0/0 (n=10) | 0/4 (n=6) | 1/1 (n=9) | 4/4 (n=6) |
| Day 7 | 0/0 (n=10) | 0/2 (n=8) | 0/1 (n=9) | 0/0 (n=10) |
| Day 14 | 0/1 (n=10) | 0/1 (n=9) | 0/0 (n=10) | 0/0 (n=10) |
| **Mucoid feces** | | | | |
| Pretest | 0/2 (n=8) | 0/3 (n=7) | 0/3 (n=7) | 0/2 (n=8) |
| Day 0 | 0/0 (n=10) | 1/4 (n=10) | 0/1 (n=9) | 0/4 (n=6) |
| Day 7 | 0/0 (n=10) | 0/2 (n=8) | 0/1 (n=9) | 0/0 (n=10) |
| Day 14 | 0/1 (n=9) | 0/1 (n=9) | 0/0 (n=10) | 0/0 (n=10) |
| **Piloerection** | | | | |
| Pretest | 0/10 | 0/10 | 0/10 | 0/10 |
| Day 0 | 0/10 | 0/10 | 0/10 | 4/10 |
| Day 7 | 0/10 | 0/10 | 0/10 | 0/10 |
| Day 14 | 0/10 | 0/10 | 0/10 | 0/10 |

Data were extracted from pages 67 to 90 of the study report.
n - number of animals for which the parameter could not be assessed (e.g., diarrhea could not be assessed in those animals which did not defecate during the observation period).

**b. Motor Activity:** Refer to Table 4. There were no treatment-related effects on motor activity. The

occasional statistically significant deviation (increased and decreased) of a single interval was noted in all dose groups. However, due to their isolated occurrence, and in the absence of a dose-response relationship, they were considered to be incidental findings unrelated to treatment.

**Table 4. Motor Activity (total activity counts for session)**

| Test Day | Dose (mg/kg bw) | | | |
|---|---|---|---|---|
| | 0 | 100 | 300 | 1000 |
| **Males** | | | | |
| Day -7 | 99±37 | 97±23 | 92±31 | 106±17 |
| Day 0 | 133±43 | 96±36 | 115±28 | 108±44 |
| Day 7 | 131±34 | 121±47 | 112±22 | 118±29 |
| Day 14 | 135±49 | 115±39 | 124±44 | 125±45 |
| **Females** | | | | |
| Day -7 | 115±40 | 106±35 | 135±30 | 131±44 |
| Day 0 | 122±33 | 89±41 | 113±22 | 102±30 |
| Day 7 | 152±60 | 132±23 | 159±46 | 140±50 |
| Day 14 | 143±46 | 165±38 | 133±35 | 159±63 |

Data were extracted from pages 91 and 92 of the study report. Values represent mean +s.d. N=10.
*p<0.05,** p<0.01 compared with controls.

6. **Sacrifice and Pathology:**

a. **Gross Pathology:** No gross lesions were observed in any animal at any dose level tested.

i. **Brain Weight:** Not measured.

b. **Neuropathology:** No treatment-related microscopic lesions were observed in any of the central or peripheral nervous system tissues examined in rats in the 1000 mg/kg bw group. The only findings were axonal degeneration in the proximal sciatic nerve observed in one control male, and dilation of the ventricles observed in 1 male in the 1000 mg/kg bw group.

## III. DISCUSSION and CONCLUSIONS:

A. **Investigators' Conclusions:** "BAS 500 F was administered to groups of 10 male and 10 female Wistar rats as a single oral administration by gavage at dose levels of 0, 100, 300 and 1000 mg/kg body weight. Body weight change was statistically significantly impaired in high dose males on day 7. A

substance-relationship cannot be excluded with certainty. Soft feces, mucoid feces, and/or diarrhea were seen in all treatment groups on the day of treatment (day 0). This was assessed as being related to treatment. As no signs of autonomic symptoms (e.g. salivation) were observed, the soft feces were assessed as being a (local) effect on the gastrointestinal tract, but not an affection of the central nervous system. Piloerection was seen in 4 high dose females on day 0. This finding was also related to the test substance administration, and demonstrates a non-specific sign of general toxicity. The light-microscopic examination of the central and peripheral nervous system did not reveal any substance-dependent changes in the organs examined. All clinical findings were observed on day 0 (day of administration) only, and were reversible by day 7 post exposure. Thus, the signs recorded in the present study were due to acute systemic toxicity and/or local effects on the digestive tract, but not to neurotoxicity. The no observed effect level for neurotoxicity under the conditions of this study was therefore 1000 mg/kg body weight in both sexes."

B. **Reviewer's Comments:** Wistar rats were given a single oral dose of BAS 500 F (purity 99.0%) in a 0.5% aqueous solution of carboxymethylcellulose at dose levels of 0, 100, 300 and 1000 mg/kg bw and observed for 14 days. Functional observational batteries (FOBs) and motor activity testing were performed in all animals at ~4 to 6 hours, 7 days and 14 days post-dosing. At study termination, 5 animals/sex/group were fixed by *in situ* perfusion and subjected to neuropathological examination. All animals survived the 14-day observation period, and there were no treatment-related clinical signs of toxicity. Decreased body weight gain was noted for males only in the 1000 mg/kg bw group during the first week of the study. Open field observations revealed an increased incidence of soft feces, mucoid feces and/or diarrhea at all dose levels on day 0 only (day of test material administration). In addition, piloerection was observed on day 0 in the high dose group, females only. However, none of these findings were considered to be toxicologically significant since they were only observed during the 2-minute open field observations, but not on day 0 or any other time during the 2 week clinical observation period.
There were no other treatment-related findings noted during the FOBs at 0, 7 or 14 days. Motor activity assessment revealed an occasional deviation of single intervals at all dose levels tested. However, the incidents were isolated and there was no dose-response relationship and so these findings were not considered to be treatment-related. There was no significant effect on the overall motor activity at any dose level tested. Gross and histopathological examination of the central nervous system and the peripheral nervous system did not reveal any treatment-related findings. The PMRA reviewer concurs with the study author that the noted treatment-related findings were due to acute systemic toxicity and/or local effects on the digestive tract.

**Based on the effects seen in this study, the LOAEL for systemic toxicity for males was 1000 mg/kg bw based on decreased body weight gain. The NOAEL was 300 mg/kg bw. The LOAEL for females could not be determined since there were no adverse, treatment-related effects. The NOAEL was 1000 mg/kg bw.**
**The LOAEL for neurotoxicity could not be determined since there were no treatment-related neurotoxic effects noted at any dose level tested. The NOAEL was 1000 mg/kg bw.**

The study is classified as acceptable as an acute neurotoxicity study in rats (870.6200, OECD 424).

C. **Study Deficiencies:** No scientific deficiencies were noted which would compromise the interpretation of the study.

13