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: Subchronic Neurotoxicity - Rats OPPTS 870.6200

DP Barcode: D269669, D267732
PC Code: 099100

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

Test Material (Purity): BAS 500 F; purity 97.09%

Synonyms: Reg. No. 304 423, Pyraclostrobin

Citation: Mellert, W., Kaufmann, W., and Hildebrand, B. (1999) BAS 500 F - Subchronic Oral Neurotoxicity Study in Wistar Rats. Department of Toxicology of BASF Aktiengesellschaft, Rhein, FRG. Laboratory Project No. 50C0494/96164, BASF Doc. No. 1999/11329, September 16, 1999. MRID # 45118401.

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

Executive Summary: In an subchronic neurotoxicity study (MRID # 45118401), BAS 500 F purity 97.09%, was administered to 10 Wistar rats/sex/group in the diet at dose levels of 0, 50, 250, and 750 (m) / 1500 (f) ppm (equal to 0, 3.5, 16.9 and 49.9 mg/kg bw/day for males, and 0, 4.0, 20.4, and 111.9 mg/kg bw/day for females) for a 3 month period. Neurobehavioral assessments (functional observation battery and motor activity testing) were performed on all animals at 22, 50, and 85 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 was 750 ppm (equal to 49.9 mg/kg bw/day) for males and 1500 ppm (equal to 111.9 mg/kg bw/day) for females based on decreased body weight gain, food intake and food efficiency (both sexes) and decreased water intake (males only). The Systemic Toxicity NOAEL was 250 ppm (equal to 16.9 mg/kg bw/day for males and 20.4 mg/kg bw/day for females. 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 750 ppm mg/kg bw (equal to 49.9 mg/kg bw/day) for males and 1500 ppm (equal to 111.9 mg/kg bw/day) for females.

This subchronic neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for a subchronic neurotoxicity study [OPPTS 870.6200(§81-8)] in rats.

EPA Reviewer Comments and Conclusions: My evaluation of the subchronic neurotoxicity study concurs with the conclusions reached by the PMRA reviewer.

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

STUDY TYPE: Subchronic Neurotoxicity Study, OPPTS 870.6200, feeding - rat; (No OECD guideline).

TEST MATERIAL (PURITY): BAS 500 F, purity 97.09%

SYNONYMS: Reg. No. 304 428, Pyraclostrobin

CITATION: Mellert, W., et al. (1999) BAS 500 F - Subchronic Oral Neurotoxicity Study in Wistar Rats Administration in the Diet for 3 Months. Department of Toxicology of BASF Aktiengesellschaft, Rhein, FRG. Laboratory Project No. 50C0494/96174, BASF Doc. No. 1999/11329, September 16, 1999. MRID #45118401. Unpublished.

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

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID #45118401), BAS 500 F, purity 97.09%, was administered to 10 Wistar rats/sex/group in the diet at dose levels of 0, 50, 250 and 750(m)/1500(f) ppm (equal to 0, 3.5, 16.9 and 49.9 mg/kg bw/day for males, and 0, 4.0, 20.4 and 111.9 mg/kg bw/day for females) for a 3 month period. Neurobehavioral assessment (functional observation battery and motor activity testing) were performed on all animals at 22, 50 and 85 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 study period, and there were no treatment-related clinical signs of toxicity. Decreased body weight gain was noted in the 750(m)/1500(f) ppm group. In addition, body weight gain was slightly decreased in the 250 ppm group, both sexes, but was not considered to be toxicologically significant (i.e., the difference in mean final body weight values compared to the control group values was < 10%). Mean food intake was decreased in the 750(m)/1500(f) ppm group. Food intake in the 250 ppm group was also slightly decreased, reflecting a marginal, treatment-related effect, but was not considered to be toxicologically significant. Food efficiency was slightly lower in the high dose group, both sexes. Water consumption was decreased in the high dose group, males only. However, in the absence of urinalysis and clinical chemistry data, the toxicological significance of this finding is uncertain. The only finding noted during the FOBs was decreased forelimb grip strength for females in the high dose group on study day 85. However, this finding was not considered to be indicative of neurotoxicity since other time intervals were not affected and since there were no other neurotoxic effects noted during the study for either sex. This finding was considered most likely to reflect normal biological variation since the values fell within the historical control range of values. 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.

Based on the effects seen in this study, the LOAEL for systemic toxicity was 750 ppm (equal to 49.9 mg/kg bw/day) for males and 1500 ppm (equal to 111.9 mg/kg bw/day) for females based on decreased body weight gain, food intake and food efficiency (both sexes) and decreased water intake (males only). The NOAEL was 250 ppm (equal to 16.9 mg/kg bw/day for males and 20.4 mg/kg bw/day for females).
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 750 ppm (equal to 49.9 mg/kg bw/day) for males and 1500 ppm (equal to 111.9 mg/kg bw/day) for females.

The study is classified as acceptable as a subchronic neurotoxicity study in rats (870.6200).

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, viscous, melt/reddish-brown, clear
Lot/Batch #:	LJ. No.27882/191/c; (Tox. III/Part 1)
Purity:	97.09 % a.i.
Compound Stability:	Proven by reanalysis after the in life phase of the study
CAS #:	175013-18-0

2. **Vehicle:** Test material dissolved in acetone, then mixed with control diet

3 **Test Animals:**

Species:	Rat	
Strain:	Wistar; Chbb:THOM (SPF)	
Age/weight at study initiation:	49 days of age Body weight: Males, 195 g to 265 g; Females, 130 g to 178 g	
Source:	Boehringer 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:	Kliba rats/mice/hamsters maintenance diet, meal, *ad libitum*	
Water:	Drinking water, *ad libitum*	
Environmental conditions:	**Temperature:**	20-24°C
	Humidity:	30-70%
	Air changes:	Not stated
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	~ 2 weeks	

B. STUDY DESIGN:

1. **In Life Dates:** August 20, 1998 to November 20, 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, ppm				
	0	50	250	750	1500
Number of males	10	10	10	10	---
Number of females	10	10	10	---	10
Behavioral Testing (FOB, Motor Activity)	10/sex	10/sex	10/sex	10 males	10 females
Neuropathology	5/sex	5/sex	5/sex	5 males	5 females

5

Doses were selected based on the results of a subchronic study (BASF 1999) in which Wistar rats were fed test diets containing BAS 500 F at concentrations of 0, 50, 150, 500, 1000 and 1500 ppm (equal to 0, 3.5, 10.7, 34.7, 68.8 and 105.8 mg/kg bw/day for males and 0, 4.2, 12.6, 40.8, 79.7 and 118.9 mg/kg bw/day for females) for a period of 3 months, 10 rats/sex/group. Reported findings were as follows:
1500 ppm: decreased food consumption and body weight gain, both sexes; increased neutrophils, MCV, reticulocyte count, prothrombin time, total bilirubin, urinary crystals and urine turbidity, both sexes; increased chloride and albumin, males only; increased WBC count, lymphocytes, MCH and urinary volume, females only; decreased hyperchromia, MCHC, ALAT, ALP, glucose and globulin, both sexes; decreased triglyceride and cholesterol, males only; decreased RBC count, Hgb, HCT, serum cholinesterase, chloride, creatinine, total protein and urine specific gravity, females only; decreased absolute adrenal weights, both sexes; decreased absolute liver and kidney weights, males only; increased absolute spleen weight, both sexes; increased absolute liver weight, females only; increased relative kidney, testes, spleen, adrenal and brain weight, males; increased relative liver, spleen, ovary and adrenal weight, females; mucosal hyperplasia of the duodenum, both sexes; increased extramedullary hematopoiesis, histiocytosis and distended sinusoids in the spleen, both sexes; and hepatocellular hypertrophy and diminishing fat storage, both sexes.
1000 ppm: decreased food intake, both sexes; decreased body weight gain, males only; increased neutrophils, MCV and prothrombin time, both sexes; increased chloride, total bilirubin, albumin and urine turbidity, males only; increased WBC count, lymphocytes, MCHC, urinary volume and urinary crystals, females only; decreased hyperchromia, MCHC, ALAT, AlkPhos, glucose and globulin, both sexes; decreased RBC count, Hgb, serum cholinesterase, chloride, creatinine, total protein and urinary specific gravity, females only; decreased absolute adrenal weight, both sexes; decreased liver weight, males only; increased absolute spleen weight, females only; increased relative kidney, testes, spleen and brain weight, males; increased relative liver, kidney and spleen weight, females; mucosal hyperplasia of the duodenum, males only; increased extramedullary hematopoiesis, histiocytosis and distended sinusoids in the spleen, both sexes; hepatocellular hypertrophy, males only; and diminishing fat storage, both sexes.
500 ppm: decreased food intake, both sexes; decreased body weight gain, males only; increased chloride and albumin, males only; increased MCV and MCH, females only; decreased ALAT, both sexes; decreased MCHC and cholesterol, males only; decreased hyperchromia, AlkPhos and glucose, females only; decreased absolute adrenal weight, both sexes; decreased absolute liver weight, males only; increased absolute spleen weight, females only; increased relative liver, kidney and spleen weight, females only; mucosal hyperplasia of the duodenum, males only; increased extramedullary hematopoiesis, histiocytosis and distended sinusoids in the spleen, both sexes; hepatocellular hypertrophy, males only; and diminished fat storage, both sexes.
150 ppm: decreased absolute liver weight, males only; and increased extramedullary hematopoiesis and histiocytosis in the spleen, both sexes.
50 ppm: no treatment-related effects.

Based on the results of this study, dose levels chosen for the 3 month rat neurotoxicity feeding study were 50 ppm as the low dose, 250 ppm as the mid dose and 750 ppm (males)/1500 ppm (females) as the high dose with expected toxic effects.

3. **Test Substance Preparation and Analysis**: Diet was freshly prepared "in intervals for which the stability of the test substance in the diet was guaranteed"and stored at room temperature. The test material was frozen and mechanically crushed, then an acetonic solution was made. The solutions were sprayed on ~ 3 kg of diet in a rotation vaporizer. The acetone was removed by heating to ~ 40°C for 30 minutes. The

premixes were then adjusted to the desired concentrations with the appropriate amount of food and mixed for 10 minutes in a GEBR.LODIGE laboratory mixer. Stability of the test material in diet prepared prior to study initiation was determined after storage at room temperature for 0 and 43 days after preparation at the dose level of 20 ppm. Homogeneity of mixing was determined for the initial batch of test diets prepared for the study at 50 and 1500 ppm. The actual test material concentration in the diets was determined for all dose levels, from samples of test diets prepared at the beginning (August 13, 1998) and at the end (October 23, 1998) of the administration period.

Results:

Stability Analysis: Test diet at the dose level of 20 ppm, after storage at room temperature for 43 days, was 104.3% of the initial concentration.

Homogeneity Analysis: Individual samples of the 50 and 1500 ppm test diets ranged from 94.8% to 98.4% and 94.7% to 95.5% of the nominal concentrations, respectively.

Concentration Analysis: The range of values for the actual concentrations of BAS 500 F in the test diets, and the overall mean values, expressed as percentage of the nominal concentrations, were as follows:

	Dose (ppm)				
	0	50	250	750	1500
Actual concentration, ppm Range of values Mean value	None detected	47.4 to 49.9 49.0	233 to 238 236	691 to 710 701	1280 to 1432 1350
% of target concentration Range of values Mean value	None detected	94.8 to 99.8 98.0	93.2 to 95.2 94.4	92.1 to 94.7 93.5	85.3 to 95.5 90.0

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, body weight change, food and water consumption, food efficiency: Parametric one-way analysis using the F-test (ANOVA). If the resulting p-value was ≤ 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 ≤ 0.05, a pairwise comparison of each dose group with the control group was performed using the two-sided Mann-Whitney U-test.
iii) Brain weight: Non-parametric one-way analysis using the two-sided Kruskal-Wallis test. If the resulting p-value was ≤ 0.05, a pairwise comparison of each dose group with the control group was performed using the Wilcoxon 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 once a week.

2. **Body Weight:** Animals were weighed on a weekly basis and on days when the functional observational batteries were performed.

3. **Food Consumption:** Food consumption was determined on a weekly basis. From the body weight and food intake data, the mean daily substance intake per animal (mg/kg bw/day) and the weekly feed efficiency per animal (weekly body weight change, g/weekly food consumption, g x 100) were calculated.

4. **Water Consumption:** Water consumption was determined on a weekly basis, starting at study week 2.

5. **Neurobehavioral Assessment:**

a. Functional Observational Battery (FOB): A functional observational battery was performed on all animals at the same time of day for each assessment, once before initiation of treatment (day -7) and on study days 22, 50 and 85. 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, 22, 50 and 85) 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.

6. 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 Narcoran and sacrificed by perfusion fixation using fixation solution according to Karnovsky. The rinsing solution used was Soerensen's phosphate buffer. Each animal was necropsied, and the brain (without olfactory bulb) was weighed.

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)
	OTHER	✓	Ventral root fiber (C3-C6)
✓	Gasserian ganglia with nerve	✓	Dorsal root ganglion (L1-L4)
	Trigeminal nerves	✓	Dorsal root fiber (L1-L4)
	Optic nerve	✓	Ventral root fiber (L1-L4)
	Eyes		
✓	Gastrocnemius muscle		

In the control and 750(m)/1500(f) ppm groups, brain, spinal cord, Gasserian ganglia and gastrocnemius muscle were embedded in paraffin, sectioned and stained with hematoxylin-eosin, then examined microscopically. In the 50 and 250 ppm groups, these tissues were preserved in 4% formaldehyde and stored. In the control and 750(m)/1500(f) ppm 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 50 and 250 ppm groups, these tissues were preserved in buffer solution and stored.

9

7. **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 baseis acceptable for use with the current study.

Historical control data were submitted for rearing, forelimb and hindlimb grip strength, landing foot 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 throughout the study period.

3. **Body Weight and Body Weight Gain:** Refer to Table 2. Mean final body weight and overall body

weight gain were lower in the high dose group, both sexes, due to lower body weight gain throughout the study period. In addition, mean final body weight and overall body weight gain were slightly lower in the 250 ppm group, both sexes, which the PMRA reviewer considered to possibly reflect a marginal, treatment-related effect. However, since the difference in mean final body weight values compared to the control group values was < 10%, this finding was not considered to be toxicologically significant.

TABLE 2. Mean Body Weights and Body Weight Gains (g)[a], 3-month treatment period

Time Interval	Dose (ppm)			
	0	50	250	750(m)/1500(f)
Males				
Week 0	228.7±18.4	227.6±15.6	224.1±21.2	223.7±14.9
Week 1	275.3±23.8	274.6±21.5	269.3±26.7	256.1±18.7
Week 4	358.3±25.3	354.2±36.0	337.3±37.6	322.4±30.3
Week 8	434.8±30.0	433.6±46.5	412.7±46.3	386.3±38.0*
Week 13 (% of control)	490.0±39.5 —	490.8±56.8 (100.2)	463.7±59.7 (94.6)	431.8±47.4* (88.1)
Bw gain, wk 0-1	46.6	47	45.2	32.4
bw gain, wk 1-4	83	79.6	68	66.3
bw gain, wk 4-8	76.5	79.4	75.4	63.9
bw gain, wk 8-13	55.2	57.2	51	45.5
Total gain (% of control)	261.3 —	263.2 (100.7)	239.6 (91.7)	208.1 (79.6)
Females				
Week 0	156.3±14.9	158.6±12.3	155.8±15.8	159.7±12.0
Week 1	173.6±18.2	179.9±12.2	172.0±20.0	166.6±10.4
Week 4	209.3±20.6	218.8±14.4	207.8±23.4	197.6±12.6
Week 8	244.9±28.5	250.2±19.8	238.3±28.4	223.2±15.1
Week 13 (% of control)	262.2±27.8 —	271.9±18.9 (103.7)	256.3±31.6 (97.7)	238.8±16.2 (91.1)
Bw gain, wk 0-1	17.3	21.3	16.2	6.9
bw gain, wk 1-4	35.7	38.9	35.8	31
bw gain, wk 4-8	35.6	31.4	30.5	25.6
bw gain, week 8-13	17.3	21.7	18	15.6

Total gain (% of control)	105.9 ----	113.3 (107.0)	100.5 (94.9)	79.1 (74.7)

[a] Data obtained from pages 73 to 82 in the study report.
* Significantly different ($p < 0.05$) from the control.

4. **Food Consumption**: Total mean food intake was lower in the high dose group, both sexes, due to decreased food intake throughout the study period. Slightly lower total mean food intake was also noted in the 250 ppm group, both sexes, which was considered to be treatment-related by the study author for males only. However, the PMRA reviewer also considers that the slightly lower food intake noted for females in the 250 ppm group could also reflect a marginal, treatment-related effect.
Total mean food intake values (daily mean value; percent of control group value in brackets) for the 0, 50, 250 and 750(m)/1500(f) ppm groups, respectively, were as follows:
i) For males: 348.3 g (26.8 g), 351.8 g (27.1 g; 101.1%), 328.9 g (25.3 g; 94.4%) and 306.3 g (23.6 g; 88.1%); and,
ii) For females: 240.3 g (18.5 g), 245.4 g (18.9 g; 102.2%), 233.0 g (17.9; 96.8%) and 204.5 g (15.7 g; 84.9%).

5. **Compound Consumption:** Based on food consumption, the nominal dietary concentrations and body weights, the doses expressed as mean daily mg test substance/kg body weight during the study period were as follows:
i) For males: 0, 3.5, 16.9 and 49.9 mg/kg bw/day for the 0, 50, 250 and 750 ppm groups, respectively; and,
ii) For females: 0, 4.0, 20.4 and 111.9 mg/kg bw/day for the 0, 50, 250 and 1500 ppm groups, respectively.

6. **Food Efficiency:** The study author concluded that there was no treatment-related effect on food efficiency. However, the PMRA reviewer considered that the slightly lower overall food efficiency in the high dose group, both sexes, could reflect a marginal, treatment-related effect, i.e., overall food efficiency values for the 0, 50, 250 and 750(m)/1500(f) ppm groups, were as follows:
i) For males, 10.5, 10.5, 10.2 and 9.7; and,
ii) For females, 6.3, 6.5, 6.0 and 5.4.

7. **Water Consumption:** Total mean water intake was lower in the high dose group, males only, due to decreased water intake throughout the study period. In the absence of urinalysis and blood biochemistry data, the toxicological significance of this finding is uncertain. The study author also considered decreased water consumption for males in the 250 ppm group during study weeks 11 to 13 to be treatment-related, since the finding was statistically significant. However, overall water consumption at this dose level was only slightly lower than the control group value, and is not considered to be toxicologically significant.
Total mean water intake values (daily mean value; percent of control group value in brackets) for the 0, 50, 250 and 750(m)/1500(f) ppm groups, respectively, were as follows:
i) For males: 291.4 g (26.5 g), 313.7 g (28.5 g; 107.5%), 271.8 g (24.7 g; 93.2%) and 230.2 g (20.9 g; 78.9%); and,
ii) For females: 203.4 g (18.5 g), 231.3 g (21.0 g; 113.5%), 192.2 g (17.5; 94.6%) and 192.2 g (17.5 g; 94.6%).

8. **Neurobehavioral Results:**

a. FOB Findings: Refer to Table 3. The only finding considered to be treatment-related was decreased grip strength noted for females in the high dose group at 85 days. The study author considered that this finding was the result of the lower body weight at this dose level, and was not indicative of neurotoxicity. The study author stated that this conclusion was supported by the fact that there were no other FOB or motor activity findings, nor any histopathological changes observed in the central or peripheral nervous systems. Examination of the individual animal data revealed that the range of grip strength values for females at each dose level were similar, i.e., for the 0, 50, 250 and 1500 ppm groups, the range of values were 5.4 to 7.6 newtons, 4.4 to 7.6 newtons, 4.6 to 7.6 newtons and 4.6 to 6.2 newtons, respectively. These data indicate that although the highest value noted in the high dose group was lower than the highest value noted at any other dose, the range of values noted in the high dose group fell within the range of values noted at the other dose levels. In addition, all values fell within the historical control range of values provided by the registrant, i.e., the range of values for rats of the same strain and age was 2.2 to 6.8 newtons, mean value of 4.0±1.0 newtons (data were collected from 26 studies, total of 70 rats). Hence, the PMRA reviewer agrees that lower forelimb grip strength for females in the high dose group is not likely indicative of neurotoxicity. However, although the study author considers this may be the result of lower body weight, it could also reflect normal variation, i.e., grip strength was not affected for high dose females on study days 22 or 50 even though body weights were lower; the grip strength of males in the high dose group was not similarly affected even though the effect on body weight was more pronounced than that noted for females; and hindlimb grip strength was not affected in the high dose group, males or females.

The only other finding was increased hindlimb grip strength for females in the 50 ppm on day 85, but was considered incidental in the absence of a dose-response relationship.

Table 3. Selected Functional Observation Battery Results -Grip Strength (newtons)

Observation	Dose (ppm)			
	0	50	250	750(m)/1500(f)
Males				
Grip Strength -Forelimbs				
Day -7	3.2±0.4	3.1±0.3	3.0±0.2	5.5±0.2
Day 22	5.6±0.9	5.8±1.0	5.8±1.1	5.6±0.5
Day 50	5.1±0.7	5.2±0.6	5.0±0.8	5.4±0.6
Day 85	5.5±0.2	5,6±0.5	5.4±0.6	5.4±0.6
Grip Strength - Hindlimbs				
Day -7	1.9±0.2	1.8±0.4	1.9±0.3	1.8±0.3
Day 22	3.9±1.0	4.0±1.2	3.9±0.9	3.7±0.8
Day 50	3.9±0.4	3.8±0.6	3.7±0.4	3.6±0.5
Day 85	4.2±0.4	4.3±0.5	4.4±0.5	3.7±0.4
Females				
Grip Strength -Forelimbs				
Day -7	1.9±0.2	2.0±0.1	2.0±0.2	2.0±0.3
Day 22	4.5±1.4	4.3±1.1	4.1±1.3	3.8±0.9
Day 50	5.1±0.9	5.3±0.7	4.8±0.5	4.8±0.6
Day 85	6.4±0.8	6.1±0.9	6.1±0.8	5.3±0.5**

Observation	Dose (ppm)			
	0	50	250	750(m)/1500(f)
Grip Strength - Hindlimbs				
Day -7	1.7±0.2	1.6±0.1	1.6±0.2	1.8±0.3
Day 22	3.5±1.3	3.2±1.2	3.2±1.3	3.1±0.9
Day 50	4.5±0.5	4.7±0.5	4.4±0.2	4.1±0.5
Day 85	4.8±0.3	5.2±0.5*	4.6±0.4	4.5±0.5

Data were extracted from pages 95 to 98 of the study report. Values represent mean ±s.d. N=10.
*p<0.05,** p<0.01 compared with controls.

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 (ppm)			
	0	50	250	750(m)/1500(f)
Males				
Day -7	113±74	109±42	111±39	92±29
Day 22	144±57	119±39	134±49	116±33
Day 50	176±43	159±40	187±42	182±53
Day 85	180±52	169±31	172±64	178±72
Females				
Day -7	128±25	152±47	136±22	155±30
Day 22	232±69	218±64	192±45	186±52
Day 50	186±46	221±99	178±34	190±48
Day 85	202±73	181±66	209±48	244±37

Data were extracted from pages 125 and 126 in the study report. Values represent mean ±s.d. N=10
*p<0.05,** p<0.01 compared with controls.

9. Sacrifice and Pathology:

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

i. Brain Weight: Refer to Table 5. There was no treatment-related effect on absolute brain weight. Relative brain weights were higher in the high dose group, reflecting the lower body weights, and were not considered to be toxicologically significant. (The relative brain weights were not calculated by the

study author; these were calculated by the PMRA reviewer using body weight measurements taken on study day 91; nimals were sacrificed and perfused on study day 92, but body weights were not measured at that time. Standard deviations were not calculated and statistical ana.lyses were not performed).

TABLE 5 - Brain Weights *, absolute (g) and relative (% body weight)

	Dose (ppm)			
	0	50	250	750(m)/1500(f)
Males - absolute	1.97±0.04	1.93±0.04	1.95±0.07	1.99±0.07
- relative	0.402	0.393	0.420	0.461
Females - absolute	1.79±0.07	1.76±0.06	1.83±0.06	1.78±0.06
- relative	0.683	0.647	0.714	0.745

* Data obtained from pages 143 and 144 in the study report.

b. Neuropathology: No treatment-related microscopic lesions were observed in any of the central or peripheral nervous system tissues examined in rats in the high dose group. The only findings were axonal degeneration in the proximal sciatic nerve observed in one control male and one high dose female, axonal degeneration in the tibial nerve observed in one high dose male and ventricular dilation of the parietal lobe observed in one high dose male. These findings were considered to be spontaneous in nature.

III. DISCUSSION and CONCLUSIONS:

A. Investigators' Conclusions: BAS 500 F was administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0, 50, 250, 750 (males) and 1500 ppm (females) for 3 months. Toxicity was characterized by impairment of food consumption (high dose males and females, mid dose males), impairment of water consumption (high dose males and females, mid dose males) and body weight/body weight change (high dose males and females). Grip strength of forelimbs was statistically significantly decreased in high dose females at the end of the study. This was assessed as being treatment-related and related to the body weight impairment at this dose level. This is confirmed by the fact that the functional observational batteries and motor activity measurement did not reveal any other signs indicative of neurotoxicity. Moreover, comprehensive microscopic investigation of the central and peripheral nervous system did not reveal any substance-dependent changes.
In conclusion, BAS 500 F caused signs of toxicity at 1500 ppm in females and 750 and 250 ppm in males. The no observed effect level under the conditions of this study was therefore 250 ppm (20.4 mg/kg body weight/day) in females and 50 ppm (3.5 mg/kg body weight/day) in males.
No signs of selective neurotoxicity were detected. The no observed effect level for neurotoxicity was therefore 1500 ppm (111.9 mg/kg body weight/day) in females and 750 ppm (49.9 mg/kg body weight/day) in males."

B. Reviewer's Comments: Male and female Chbb:THOM (SPF) Wistar rats were fed test diets containing BAS 500 F (purity 97.09%) at dose levels of 0, 50, 250 and 750(m)/1500(f) ppm (equal to 0, 3.5, 16.9 and 49 mg/kg bw/day for males and 0, 4.0, 24.0 and 111.9 mg/kg bw/day for females) for a period of 3 months, 10 rats/sex/group. Functional observational batteries (FOBs) and motor activity testing were performed in all animals on study days 22, 50 and 85. At study termination, 5 animals/sex/group were fixed by *in situ* perfusion and subjected to neuropathological examination. All

animals survived the study period, and there were no treatment-related clinical signs of toxicity. Decreased body weight gain was noted in the 750(m)/1500(f) ppm group. In addition, body weight gain was slightly decreased in the 250 ppm group, both sexes, but was not considered to be toxicologically significant (i.e., the difference in mean final body weight values compared to the control group values was < 10%). Mean food intake was decreased in the 750(m)/1500(f) group. Food intake in the 250 ppm group was also slightly decreased (i.e., 94.4% and 96.8% of the control group values for males and females, respectively) and was considered to reflect a marginal, treatment-related effect. Food efficiency was slightly lower in the high dose group. Water consumption was decreased in the high dose group, males only. However, in the absence of urinalysis and blood biochemistry data, the toxicological significance of this finding is uncertain. The only finding noted during the FOBs was decreased forelimb grip strength for females in the high dose group on study day 85. However, this finding was not considered to be indicative of neurotoxicity since there were no other neurotoxic effects noted during the study, and was considered to reflect normal biological variation since values fell within the range of historical control values supplied by the registrant. The study author considered this effect was possibly related to the lower body weight noted at this dose level; however, a similar effect was not noted for high dose males even though the effect on body weight gain was more pronounced than that noted for females, and hindlimb grip strength was not affected for males or females at any time interval). 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.

Based on the effects seen in this study, the LOAEL for systemic toxicity was 750 ppm (equal to 49.9 mg/kg bw/day) for males and 1500 ppm (equal to 111.9 mg/kg bw/day) for females based on decreased body weight gain, food intake and food efficiency (both sexes) and decreased water consumption (males only). The NOAEL was 250 ppm (equal to 16.9 mg/kg bw/day for males and 20.4 mg/kg bw/day for females).
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 750 ppm for males (equal to 49.9 mg/kg bw/day) and 1500 ppm for females (equal to 111.9 mg/kg bw/day).

The study is classified as acceptable as a subchronic neurotoxicity study in rats.

C. <u>Study Deficiencies</u>: No scientific deficiencies were noted which would compromise the interpretation of the study.