DATA EVALUATION RECORD

BAS 510 F

STUDY TYPE: ACUTE NEUROTOXICITY - RAT
(OPPTS 870.6200a/OECD 424)
MRID 45404820

Prepared for

1/23/2002

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

Prepared by

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Task Order No. 02-06

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DATA EVALUATION RECORD
TXR#: 0050193

STUDY TYPE: Acute Neurotoxicity - Rats OPPTS 870.6200a [§81-8]; OECD 424.

PC CODE: 128008 **DP BARCODE:** D278384
SUBMISSION NO.: S604279

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

SYNONYMS: None provided

CITATION: Mellert, W., W. Kaufmann, and B. Hildebrand (2000) BAS 510 F: Acute oral neurotoxicity study in Wistar rats. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, D-67056 Ludwigshafen/Rhein, FRG. Report Project Number 20C0179/97144, BASF Registration Document Number 2000/1018638, November 9, 2000. MRID 45404820. Unpublished.

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

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID 45404820), groups of 10 male and 10 female 49-day old Wistar rats were given a single oral dose of BAS 510 F (96.3% a.i., batch N 46) in 0.5% carboxymethyl cellulose at doses of 0, 500, 1000, or 2000 mg/kg bw and observed for 14 days. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in 10 animals/sex/group on days -7, 0, 7, and 14. Cholinesterase activities were not measured. At study termination, 5 animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Of the perfused animals, 5 animals/sex in the control and 2000 mg/kg bw groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

There were no effects on mortality, body weight, motor activity, or clinical signs outside of the FOB. During the FOB, piloerection was observed in 2 female rats in the 2000 mg/kg dose group on day 0. No other FOB findings in either sex in any dose group were considered treatment-related. There were no gross organ lesions and no microscopically-observed lesions of the brain or peripheral nervous system tissues. Although the NOAEL for systemic effects in this study is 1000 mg/kg bw, in the absence of clinical signs related to neurotoxicity and in the absence of lesions of the brain and peripheral nervous system, the NOAEL for acute neurotoxicity is 2000 mg/kg bw.

Based on the effects seen in this study, the systemic LOAEL for BAS 510 F was 2000 mg/kg bw/day (based on piloerection in females), with a NOAEL of 1000 mg/kg bw/day.

The neurotoxicity LOAEL was > 2000 mg/kg bw/day and the NOAEL was 2000 mg/kg bw/day.

This neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424).

COMPLIANCE: Signed and dated GLP (German and OECD), Flagging Criteria, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

B. MATERIALS:

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

Description:	solid white material, considered stable
Lot/Batch #:	N 46
Purity:	96.3% a.i.
CAS # of TGAI:	188425-85-6
Structure:	Not provided

2. **Vehicle and/or positive control:** Vehicle: 0.5% carboxymethyl cellulose (Hoechst AG, Frankfurt/Main, Germany) in doubly distilled water.

3. **Test animals:**

Species:	Rat	
Strain:	Wistar [Chbb:THOM SPF)]	
Age/weight at dosing:	49 days old; males: 220-268 g (mean, 243g); females: 133-188 g (mean, 164 g).	
Source:	Boehringer Ingelheim Pharma KG	
Housing:	singly, in type DK III stainless steel wire cages (Becker & Co., Castrop-Rauxel, Germany)	
Diet:	Ground Kliba maintenance diet rat/mouse/hamster pellets (Klingentalmuhle AG, Kaiseraugst, Switzerland), *ad libitum*	
Water:	water bottles, *ad libitum*	
Environmental conditions:	**Temperature:**	20-24°C
	Humidity:	30-70%
	Air changes:	Not provided; room was air conditioned
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	14 days	

B. STUDY DESIGN:

1. **In life dates** - Start: December 28, 1998 End: January 15, 1999

2. **Animal assignment and treatment** - Animals were assigned to the test groups noted in Table 1 by a computerized random sort program so that body weight means for each group were comparable. Because testing occurred on 4 consecutive days, the study was conducted with 4 subsets, each of which was the same age on the day of treatment. Each subset was

composed of either males (2 subsets, 5 males from each of the dose groups) or females (2 subsets, 5 females from each dose group). Rats were given a single dose by gavage in 0.5% carboxymethyl cellulose in 20 mL/kg body weight then observed twice a day on weekdays (once/day on weekends) for 14 days. An overnight fast prior to dosing was not mentioned. Animals were weighed weekly for 14 days. Dose levels were chosen based on a peak range-finding study in which groups of 3 male and 3 female rats were administered the test substance at dose levels of 1000 or 2000 mg/kg bw. During the first day, animals were observed at 15 and 30 minutes post-dosing and then hourly for up to 6 hours. No abnormal clinical symptoms were observed at these times or during days 2 and 3. Therefore, the 2000 mg/kg dose was chosen as the high dose in the present study, with one-half and one-fourth of the high dose chosen for the mid and low doses. As no clinical signs were observed during the 3 days following dosing, a "time of peak effect" could not be determined. Therefore, the time of neurotoxicity testing was started at 3 hours after gavage. As noted, administration was staggered over a 4-day interval to facilitate neurobehavioral observations. Males and females were treated on separate days. The first 5 animals/group were sacrificed and a necropsy was performed on all animals. Five animals/sex from the control and high-dose groups were examined for microscopic lesions of the central and peripheral nervous systems. Blood and brain cholinesterase activities were not measured.

TABLE 1. Study Design

Experimental Parameter	Dose Group (mg/kg bw)			
	0	500	1000	2000
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

3. **Test substance preparation and analysis**: The test substance was prepared in doubly distilled water containing 0.5% carboxymethyl cellulose (Tylose®) CB30000. The appropriate amount of test material for each dose group was weighed and added to an appropriate amount of distilled water followed by addition of the appropriate volume of carboxymethyl cellulose to make a 0.5% solution. The solutions were mixed with a magnetic stirrer; mixing continued during the administration period. Samples to be analyzed for homogeneity were taken from the bottom, middle and top of the low- (2.5 g/100 mL) and high-dose (10.0 g/100 mL) suspensions at the start of the administration period. These samples and additional samples taken from the middle of the mid-dose suspension (5 g/100 mL) served as samples for concentration analyses. Samples were stored frozen prior to analysis. Prior to study initiation, two samples were taken from solutions containing 50 mg/100 mL and 20 g/100 mL and stored at ambient temperature for 96 hours. These samples were used for stability analysis. Samples were quantified by HPLC separation/UV detection.

Results -

Homogeneity analysis: Samples from the top, middle, and bottom of the 2.5 g/100 mL solution were 97.6, 98.0, and 98.0% of nominal. Samples from the 10.0 g/100 mL solution

were 101.0, 102.0, and 102.0% of nominal. Respective mean values for the two concentrations were 97.9±0.2 and 101.7±0.6%.

Stability analysis: At 4 and 96 hours after storage at ambient temperature, the concentrations of the 50 mg/100 mL sample were 105.1 and 96.8% of nominal, respectively. Respective values for the 20 g/100 mL sample were 103.9 and 91.0%.

Concentration analysis: Mean concentrations of the 2.5, 5.0, and 10 g/100mL solutions were 97.9±0.2, 102, and 101.7±0.6% of nominal, 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: For body weight and body weight change, a parametric ANOVA using the F-test was performed. If the resulting p value was equal to or less than 0.05, a comparison of each group with the control group was made using Dunnett's test (two sided). For FOB parameters such as feces, rearing, forelimb and hindlimb grip strength, and landing foot splay and for motor activity, non-parametric one-way analysis using the two-sided Kruskal-Wallis test was used. If the resulting p value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using the two-sided Mann-Whitney U-test for equal medians. The Reviewer considers the analyses used to be appropriate.

C. METHODS / OBSERVATIONS:

1. Mortality and Clinical observations: Animals were inspected twice a day during the week and once a day on the weekends and holidays for mortality and morbidity. Detailed physical examinations were performed daily except during days when the FOB was performed.

2. Body weight: Animals were weighed on days -7, 0, 7 and 14.

3. Food consumption: Food consumption was not recorded.

4. Cholinesterase determination: Cholinesterase activity was not determined.

5. Neurobehavioral assessment:

a. Functional observational battery (FOB): The FOB consisted of 4 parts: home cage observations, open field observations, sensorimotor tests, and reflex tests. The examinations were carried out by trained technicians who had also performed positive control studies as part of their training. Technicians were blind to the treatment of the animals and the animals were tested in a randomized order. Results were summarized by a second technician. Where appropriate, the tested parameter was ranked for severity. The open field testing was performed in a standard arena (50 x 50 x 25 cm); the observations lasted at least 2 minutes. The environmental conditions were not further described. Grip strength was measured with a newtonmeter (not further described). The FOB was performed at approximately 3 hours after dosing and at the same time of day on days 7 and 14.

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

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*	X	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*
X	Palpebral closure*	X	Palpebral closure*	X	Abnormal movements*
X	Feces consistency	X	Respiratory rate+	X	Urination / defecation*
		-	Red/crusty deposits*	-	Grooming
	SENSORY OBSERVATIONS	X	Mucous membranes /eye /skin color	X	Gait abnormalities / posture*
X	Approach response+	X	Eye prominence*	X	Gait score*
X	Touch response+	-	Muscle tone*	-	Bizarre / stereotypic behavior*
X	Startle response*			-	Backing
X	Pain response*			-	Time to first step
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	Pinna reflex		OTHER OBSERVATIONS	-	Rotarod performance

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

b. **Locomotor activity:** Locomotor Activity was evaluated following the FOB (approximately 6 hours after dosing). Animals were tested in the dark with a Multi-Varimex-System (Columbus Instruments). Interruptions of 4 infrared beams were counted over 12 five-minute intervals, indicating total activity. There were two replicates for each sex.

6. **Sacrifice and pathology:** The first 5 animals per sex and group were anesthetized (Narcoren®) and sacrificed by perfusion fixation. The remaining animals were sacrificed under CO_2 anesthesia and discarded. The following peripheral nervous system tissues from the control and 2000 mg/kg groups were embedded in epoxy resin, sectioned, and stained with Azure II-methylene blue-basic Fuchsin for light microscopic examination: cervical and lumbar ganglia and root fibers and the sciatic, tibial and sural nerves. Tissues from the 500 and 1000 mg/kg groups were stored in phosphate buffer solution. The remaining organs and tissues of the control and 2000 mg/kg dose groups were embedded in paraffin, thin-sectioned, and stained with hematoxylin-eosin for light microscopic examination. Tissues from the 500 and 1000 mg/kg groups were preserved in 4% formaldehyde. Brain weight and brain measurements were not recorded.

The CHECKED (X) tissues from the control and 2000 mg/kg dose groups were evaluated microscopically. "-" refers to not taken for evaluation.

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	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		SCIATIC NERVE
X	Forebrain	X	Proximal
X	Center of cerebrum	-	Sciatic Notch
X	Midbrain		
X	Cerebellum		OTHER
X	Pons	X	Sural Nerve
X	Medulla oblongata	X	Tibial Nerve
	SPINAL CORD	-	Peroneal Nerve
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Lumbar swelling	X	Lumbar dorsal root fibers
-	Thoracic swelling	X	Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
X	Gasserian Ganglion	X	Cervical dorsal root fibers
-	Trigeminal nerves	X	Cervical ventral root fibers
-	Optic nerve		
-	Eyes		
X	Gastrocnemius muscle		

7. **Positive controls:** Positive control data providing evidence of the observational methods to detect major neurotoxic endpoints and increases and decreases in activity were provided in summary form in Volume III. Repeated doses of acrylamide (40 mg/kg) to rats provided evidence of the ability to detect clinical signs and limb weakness. Microscopically, central and peripheral nervous system lesions were detected (figures of the stained sections of the cortex, tibial nerve, and gastrocnemius muscle nerve were provided). Injections of trimethyltin chloride resulted in clinical signs of ataxia, convulsions, reduced grip strength, and central nervous system pathology. Signs and pathology following treatment with 3,3'-iminodiproprionitrile and carbaryl were listed, but data were not provided. It was stated that tests with carbaryl are undertaken about once/year. Data in the form of graphs were provided demonstrating an increase in motor activity associated with nomifensin and a decrease in activity associated with diazepam. Historical control data for rearing, grip strength, foot splay, and motor activity were also provided in summary tables. However, concurrent control data were used for the present study. The positive data, although undated, were acceptable as demonstrations of the ability to detect various endpoints. It was stated that several of the studies demonstrated interobserver reliability of the technicians, but data for different technicians were not provided.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical signs** - No treatment-related clinical signs were observed.

2. **Mortality** - No animals died during the study period.

B. **BODY WEIGHT AND BODY WEIGHT GAIN:** There were no treatment-related effects on body weight. At both 7 and 14 days after treatment, group mean weights for males and

females in the 2000 mg/kg treatment group were 100-101%, of the respective mean control weights (Table 2). Body weight gains were not affected by treatment.

TABLE 2. Body weight and body weight gain (g)

Observation	Dose Level (mg/kg bw)			
	0	500	1000	2000
Body weight-Males				
Day 0	243.7±9.5	244.3±13.3	241.3±12.0	242.4±9.7
Day 7	289.2±12.2	290.4±17.1	282.9±11.9	289.1±10.0
Day 14	318.5±14.3	320.7±20.8	313.2±13.9	322.7±13.2
Body weight-Females				
Day 0	167.6±11.4	162.1±15.9	165.2±8.9	161.4±11.9
Day 7	181.8±13.5	181.0±21.2	181.4±8.3	182.5±16.0
Day 14	201.3±19.8	198.5±22.5	194.1±13.2	201.1±17.3
Body weight gain-Males				
Days 0-7	45.5	46.1	41.6	46.7
Days 0- 14	74.8	76.4	71.9	80.3
Body weight gain-Females				
Days 0-7	14.2	18.9	16.2	21.1
Days 0-14	33.7	36.4	28.9	39.7

Data were extracted from Tables IA 003-IA 006, pp. 58-61, MRID 45404820.
Values represent mean ± s.d.
n = 10.

C. **FOOD CONSUMPTION**: Data on food consumption were not provided.

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

E. **NEUROBEHAVIORAL RESULTS:**

1. **FOB Findings:** On the day of treatment, piloerection was observed in two females in the 2000 mg/kg dose group. This effect was not present in any other female dose group at any other observation time or in males in any dose group at any observation time. As no other treatment-related clinical signs were observed in this group, the significance of piloerection is unclear. On day 7, forelimb grip strength was decreased in males in the 2000 mg/kg group (control, 6.6 newtons; 2000 mg/kg, 5.7 newtons; $p<0.002$). This effect was not present on days 0 or 14, but mean values for this group ran slightly lower than the control group values on other days. Because the mean value was comparable to values in all other groups during most testing periods, the decrease in forelimb grip strength does not appear to be a treatment-related effect. No additional treatment-related clinical or neurological signs were observed in any group.

2. **Motor activity:** Total motor activity for the 60-minute session, measured as infrared beam interruptions did not differ among groups (Table 3). A statistically significant increase in total activity for females in the 1000 mg/kg group on day 7 was not dose-related and was

considered incidental to treatment. For this group on day 7, activity in each of the 12 sessions was slightly higher than that of the control group, with statistical significance attained ($p<0.01$) for only session 10. For most groups on most days, habituation was reached by session 6.

TABLE 3. Motor activity [total activity counts (beam interruptions) for session]

Test Day	Dose Level (mg/kg bw)			
	0	500	1000	2000
Males				
Pre-test	131±40	101±30	129±26	141±62
Day 0	146±63	162±94	175±88	154±104
Day 7	122±48	108±23	109±22	110±24
Day 14	121±33	117±34	116±23	130±33
Females				
Pre-test	176±54	199±46	199±71	234±84
Day 0	124±24	179±57	160±67	143±43
Day 7	150±34	187±44	212±55**	174±34
Day 14	153±63	180±52	167±44	186±39

Data were extracted from Tables IA 041 and IA 042, pp. 96-97, MRID 45404820.
Values represent mean ±s.d.
n = 10 animals.
** $p<0.02$ compared with controls.

F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** No gross lesions were observed.

2. **Brain weight:** Brains were not weighed.

3. **Neuropathology:** No lesions were found in any dose group.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The authors concluded that piloerection in 2 females in the 2000 mg/kg dose group was a sign of general toxicity and not neurotoxicity. This effect was not observed in males in the 2000 mg/kg dose group or in either sex in any other dose group. There were no treatment-related neurological effects observed during the FOB and motor activity evaluations, and light-microscopic evaluation failed to reveal lesions of the central or peripheral nervous system in any animals. Therefore, based on general toxicity, the LOAEL in female rats was 2000 mg/kg bw; a LOAEL was not attained in male rats. The respective NOAELs in male and female rats were 2000 and 1000 mg/kg bw. The NOAEL for neurotoxicity in both male and female rats was 2000 mg/kg bw.

B. REVIEWER COMMENTS: Following treatment of male and female Wistar rats with single gavage doses of BAS 510 F at 0, 500, 1000, or 2000 mg/kg bw, there were no effects on body weight or motor activity. The only treatment-related clinical sign observed during the FOB was piloerection on day 0 in 2/5 females in the 2000 mg/kg bw group. This effect was considered treatment-related. There were no gross organ lesions and no microscopic lesions of the central or peripheral nervous system. The data on microscopic lesions of the nervous systems was presented in summary tables without details. However, data presented under the positive control section (Volume III) indicated that the investigators are capable of observing treatment-related lesions. The Reviewer agrees with the Investigators' conclusions concerning a LOAEL and NOAEL for the study.

For systemic toxicity, the LOAEL for BAS 510 F in female rats is 2000 mg/kg based on piloerection; a LOAEL was not attained in male rats. The NOAELs for male and female rats are 2000 and 1000 mg/kg, respectively.

For neurotoxicity, the LOAELs for both sexes were > 2000 mg/kg and the NOAELs were 2000 mg/kg.

C. STUDY DEFICIENCIES: There were no study deficiencies. At several places in the study such as the Summary and Discussion and Conclusion sections, the doses were mistakenly listed as 0, 100, 300, and 2000 mg/kg. Some of the FOB data tables were difficult to interpret, particularly where an observation could not be made; for example, the inability to observe gait in the home cage because the animals were not moving. These numbers could be mistakenly read as an effect rather than the number of animals not observed to move. The batch number of the material tested for stability was listed as N 26, whereas, the batch number of the test material used in the study and tested for concentration and homogeneity was listed as N 46.

DATA FOR ENTRY INTO ISIS

Acute Neurotoxicity Study - rats (870.6200a)

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	45404820	acute neurotoxicity	rats	1 dose	oral	gavage	500-2000	0, 500, 1000, 2000	1000	2000	systemic-piloerection	