DATA EVALUATION RECORD

BAS 510 F

STUDY TYPE: HEPATIC ENZYME INDUCTION IN WISTAR RATS

MRID 45404902

7/24/2002

Prepared for

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
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Task No. 02-06

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DATA EVALUATION RECORD
TXR#: 0050193

STUDY TYPE: Special Study, Hepatic Enzyme Induction in Wistar Rats

PC CODE: 128008

DP BARCODE: D278384
SUBMISSION NO.: S 604279

TEST MATERIAL (PURITY): BAS 510 F (95.3%)

SYNONYMS: Reg. No. 300 355, 2-chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide (IUPAC)

CITATION: Mellert, W., Kaufman, W. Leibold, E., et. al. (1999). BAS 510 F - Hepatic enzyme induction study in Wistar rats; administration in the diet for 2 weeks. Dept. Toxicology of BASF Aktiengesellschaft, D-67056 Ludwigshafen/Rhein, Germany. BASF Reg. Doc. No. 1999/10522, March 23, 1999. Laboratory Project Identification 99C0179/97063. MRID 45404902. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, NC 27709-3528.

EXECUTIVE SUMMARY: In a study done to investigate hepatic enzyme induction (MRID 45404902) BAS 510 F (95.3% a.i., lot no. Tox Charge II/N26) was administered to groups of 5 male and 5 female Wistar Chbb:THOM (SPF) rats in the diet at concentrations of 0 or 15,000 ppm (equivalent to 0 or ~1500 mg/kg bw/day) for two weeks. The rats were then killed, the livers removed, weighed, and homogenized for total and subfamily cytochrom P450 (CYP450) activity, glutathione concentration, and evidence of lipid peroxidation. Groups of 3 male and 3 female rats were similarly treated for two weeks, after which the animals were killed and the lobus dexter medialis portion of the liver prepared for light and electron microscopy.

Following treatment for two weeks, hypertrophy of zone III hepatocytes was found in male and female rats fed 15,000 ppm BAS 510 F in the diet. This was indicated by a >20% increase in liver weight of both sexes, a significant increase in total liver homogenate CYP450 activity, and slight to extensive microscopic SER proliferation without alteration of mitochondrial cristae. To determine which family of enzymes was responsible for the induction of total CYP450 activity, a series of enzyme studies was done.

No increase in the cyanide insensitive β-oxidation of palmatoyl-CoA was found indicating that the test material was not a peroxisome proliferator and that the activities of enzymes in the CYP450 4A subfamily were likely not induced. In addition, no notable microscopic increase in the size or number of peroxisomes was found. The activities of the CYP450 1A subfamilies were not increased as indicated by no increase in the *O*-dealkylation of ethoxyresorufin by male

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or female rats. However, the *O*-dealkylation of pentaoxyresorufin by CYP450 2B4 was approximately double in 15,000 ppm male rats relative to control male rats while no significant increase was found in female rats. This is likely a secondary effect from the induction of another CYP450 subfamily since the increase is not of the magnitude expected. No decrease in cellular glutathione concentration was found although lipid peroxidation was slightly increased in 15,000 ppm male rats.

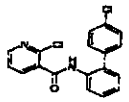
The study results suggest that BAS 510 F is an inducer of total CYP450 activity, although the subfamily responsible for the increase was not identified. The study is considered **acceptable/ nonguideline** for the investigation of hepatic enzyme induction in Wistar rats following treatment with BAS 510 F.

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

I. MATERIALS AND METHODS

A. MATERIALS:

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

Description:	White powder
Lot/Batch #:	Tox Charge II/N 26
Purity:	95.3%
Compound Stability:	Proven stable by reanalysis following in-life portion of study
CAS # if TGAI:	188425-85-6
Structure	

2. **Vehicle:** diet

3. **Test animals:**

Species:	Rat	
Strain:	Wistar Chbb:THOM (SPF)	
Age/weight at study initiation:	42 Day old; males, 180-199 g; females, 134-168 g	
Source:	Dr. Karl Thomae GmbH, Biberach/Riss, Germany	
Housing:	Singly in type DK III stainless steel wire mesh cages	
Diet:	Ground Kliba maintenance diet rat/mouse/hamster, meal, supplied by Provimi Kliba SA, Kaiseraugst, Switzerland; *ad libitum*	
Water:	*ad libitum* (water bottles)	
Environmental conditions:	**Temperature:**	20-24°C
	Humidity:	30-70%
	Air changes:	Not specified, but animal room was fully air-conditioned
	Photoperiod:	12 hrs light/dark
Acclimation period:	9 days	

B. STUDY DESIGN:

1. **In life dates:** Start: October 29, 1997; End: November 12, 1997

2. **Animal assignment:** Animals were assigned by weight using computer randomization to the test groups noted in Table 1.

TABLE 1. Study Design

Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg bw/day)		Number of animals	
		Male	Female	Male	Female
00	0	0	0	5	5
01	15,000	1507	1494	5	5
10	0	0	0	3	3
11	15,000	1405	1556	3	3

3. **Dose selection rationale:** The study authors stated the toxicity of the test substance was very low; therefore, 15,000 ppm test material in the diet was used. The study was done to determine hepatic enzyme induction.

4. **Diet preparation and analysis:** An appropriate amount of the test material was weighed and mixed throughly with a small amount of diet in a beaker. The premix was added to an appropriate amount of diet and mixed in a GEBR. LÖDGE mixer for 10 minutes to create the 15,000 ppm diet, the only concentration used in the study. Diets given to the test animals were changed weekly.

Results:

Homogeneity Analysis: The adequacy of the mixing procedure to produce a homogenous dietary mixture was confirmed in earlier studies.

Stability Analysis: Stability was confirmed in earlier studies. These showed that the test material was stable in the diet at room temperature for at least 32 days.

Concentration Analysis: Diet concentration of the test material was within 3.5% of nominal.

5. **Statistics:** Comparisons of treated versus control for body weight, food consumption, body weight change, and food efficiency were done by the Students t-test (two-sided). Clinical chemistry results were compared by the non-parametric Mann-Whitney or Wilcoxon tests. The level of significance for all statistical analyses was $p \leq 0.05$. The reviewer considered the analyses appropriate for the study.

C. METHODS:

1. **Observations:**

1a. **Cageside observations:** Animals were inspected twice daily for signs of toxicity and mortality except on weekends or holidays when a single inspection was done.

1b. **Clinical examinations**: Detailed clinical examinations were conducted daily.

2. **Body weight**: Animals were weighed at the start of the study, on day 7, and at sacrifice.

3. **Food consumption and compound intake:** Food consumption was determined weekly as a representative value over the 7-day period.

4. **Sacrifice:** Five male and five female rats per treatment group were sacrificed by decapitation under CO_2 anesthesia two weeks after the start of the study. The livers of the rats were perfused *in situ* with 0.9% saline (about 20 ml) through the portal vein, removed, and weighed. The livers were then homogenized (procedure not described). An additional 3 male and female rats/group were anesthetized with nembutal and the animals sacrificed by perfusion fixation with 5% glutaraldehyde in cacodylate buffer. The lobus dexter medialis was removed and processed for semi-thin and ultra-thin sections that were later examined by light and electron microscopy.

5. **Clinical bioanalytics:** *Cyanide-insensitive palmitoyl-CoA-oxidation* - The method, as described by Lazarow [Lazarow, P., 1981, Enzym. 72, 315-319], follows the four enzyme cascade of fatty acid β-oxidation. The reaction was monitored kinetically at 334 nm.

   Total protein - Total protein of the liver homogenate was determined by the biuret method.

   Glutathione - Glutathione concentration was determined spectrophotometrically according to the method of Sedlak, J. and Lidsay, R. as described in Anal. Biochemistry 25, 192-205 (1968). The reaction was monitored at 412 nm.

   Total cytochrome P450 (CYP450) content - CYP450 content was measured by differential absorption at 450 nm and 490 nm as described by Omura and Sato in the J. Biol. Chem., 239, 2370-2378 (1964).

   Ethoxyresorufin-O-deethylase (EROD) and pentoxyresorufin-O-dependylase (PROD) activities - EROD and PROD activities were measured fluorimetrically at an excitation wavelength of 550 nm and an emission wavelength of 585 nm according to the method of Lubet et. al., as described in Chem.Biol. Interactions, 75, 325-339 (1990).

   Lipid peroxidation - Lipid peroxidation was measured by following malonaldehyde's reaction with thiobarbituric acid spectrophotometrically at 535 nm as described by Preece et al. in Toxicol. Appl. Pharmacol 93, 89-100 (1988).

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity**: No clinical signs of toxicity were observed.

2. **Mortality**: None of the animals died during the study.

B. **BODY WEIGHT AND WEIGHT GAIN**: No biologically relevant effects on body weight were found.

C. **FOOD CONSUMPTION AND COMPOUND INTAKE:** No biologically relevant effects on food consumption or food efficiency were found. Actual compound intake is shown in Table 1 above.

D. **SACRIFICE AND PATHOLOGY:**

1. **Organ weight**: The absolute liver weight of treated rats was statistically increased 33% in males and 23% in females within two-weeks of treatment with the test material (Table 2). Likewise, the liver to body weight ratios for male and female rats were increased 26% and 20%, respectively.

TABLE 2. Absolute weight (g) and liver to body weight (%) of male and female rats treated with BAS 510 F for two weeks [a]

Dose (ppm)	Absolute Liver Weight (g)		Liver to Body Wt. (%)	
	Males	Females	Males	Females
0	13.5 ± 1.21	8.8 ± 0.73	4.7 ± 0.19	4.6 ± 0.27
15,000	17.9*** ± 0.77	10.8*** ± 0.82	6.2** ± 0.62	5.5*** ± 0.22

[a] N=5 for all groups
** = p≤0.01; *** = p≤0.001 as calculated by reviewer using Students t-test
Data from p 66, MRID 45404902 and from pp 97, 99, & 103

2. **Liver pathology**: There were no grossly observable lesions and no treatment-related effects found with light microscopy. Electron microscopy, however, showed slight to extensive smooth endoplasmic reticulum (SER) proliferation in zone three (centrilobular) hepatocytes. Glycogen depletion was also observed in hepatocytes with extensive SER proliferation. No increases in the number or size of peroxisomes or increases or decreases in mitochondrial cristae were observed.

3. **Liver homogenate bioanalytics**: *Cyanide insensitive palmatoyl-CoA oxidation* - No treatment-related increase in the oxidation of palmatoyl-CoA was found, indicating no peroxisome proliferation occurred.

EROD and PROD activity - EROD and PROD activities were not statistically increased in liver homogenates of treated male and female rats, although, PROD activity in 15,000 ppm males was approximately double that of their respective control rats (Table 3).

Glutathione concentration - The concentration of glutathione in the liver homogenates was unaffected by treatment, suggesting no increase in the formation of metabolic reactive intermediates.

Lipid peroxidation - The concentration of MDA equivalents in the liver homogenates of treated male rats was statistically increased 105%; however, the concentration of MDA equivalents was not significantly increased in treated female rats (Table 3).

Total CYP450 content - Total CYP450 content was increased 124% in treated males and 74% in treated females (Table 3).

TABLE 3. Concentration or activity of various homogenate constituents in the liver of male and female rats that received 0 ppm or 15,000 ppm BAS 510 F for two weeks.

Constituent	Males		Females	
	Dose (ppm)			
	0	15,000	0	15,000
EROD (nmol/min/mg prot.)	2.18 ± 1.85	3.30 ± 1.79	3.10 ± 0.56	3.26 ± 2.37
PROD (nmol/min/mg prot.)	4.33 ± 1.46	8.02 ± 2.26	3.25 ± 0.70	4.12 ± 1.46
Total CYP450 (nmol/mg prot.)	0.145 ± 0.01	0.325** ± 0.114	0.084 ± 0.019	0.146** ± 0.017
Lipid Peroxidation [MDA-eq (nmol/g tissue)]	9.8 ± 6.7	20.1** ± 5.4	1.0 ± 0.2	0.8 ± 0.9

**$p \leq 0.01$
n = 5 for all groups except female high-dose lipid peroxidation rats where n = 4
Data taken from pages 67-71 of MRID 45404902

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** According to the study author, treatment of male and female Wistar rats with 15,000 ppm BAS 510 F for two weeks did not induce peroxisome proliferation as indicated by no significant increase in fatty acid oxidation or an increase in the visual number of peroxisomes. While total CYP450 activity was increased in both sexes, the CYP450-related activities of the EROD and PROD were not induced by treatment. Although no treatment-related changes were seen in cellular concentrations of glutathione in either sex, lipid peroxidation was slightly increased in male rats. Proliferation of hepatocellular SER was seen in both sexes of rats, however the number and size of mitochondria were not altered. On the basis of these results, the author concluded that BAS 510 F was an inducer of rat liver CYP450.

B. **REVIEWER COMMENTS:** In this study, hepatocellular hypertrophy resulted from feeding male and female rats 15,000 ppm BAS 510 F for two weeks. This was indicated by the >20% increase in liver weight of males and females, a significant increase in total liver homogenate CYP450 activity, and slight to extensive microscopic SER proliferation without alteration of mitochondrial cristae. To determine which family of enzymes was responsible for the centrilobular hepatocellular hypertrophy with induction of total CYP450 activity, a series of enzyme studies was done.

The first of these followed the cyanide insensitive four enzyme cascade of palmatoyl-CoA β-oxidation by peroxisomes. No increase in the β-oxidation of the fatty acids was found indicating the test material is not a peroxisome proliferator. This was substantiated by no notable microscopic increase in the number or size of hepatic peroxisomes. These results

also suggest that the activities the CYP450 4A subfamily of enzymes responsible for fatty acid ω- and ω-1 oxidation were not induced.

The study results also indicate that the activities of the CYP450 1A subfamilies were not increased as indicated by no increase in the *O*-dealkylation of ethoxyresorufin by treated male or female rats. However, the *O*-dealkylation of pentaoxyresorufin was approximately double in 15,000 ppm male rats but not female rats relative to their respective controls. This suggests that the CYP450 2B4 subfamily may be slightly increased following treatment with BAS 510 F; however, the reviewer thinks this is likely secondary to the induction of another CYP450 subfamily since the increase was not of the magnitude expected relative to total CYP450 activity.

Although no decrease in cellular glutathione concentration was found, lipid peroxidation was slightly increased in 15,000 ppm male but not female rats. These results suggest that the increase in lipid peroxidation is ambiguous. Therefore, the reviewer agrees with the study author that BAS 510 F is an inducer of total CYP450 activity, although the subfamily responsible for the increase was not identified. The study is considered **acceptable/ nonguideline** for the induction of hepatic enzymes in Wistar rats following treatment with BAS 510 F.

C. **STUDY DEFICIENCIES:** No significant study deficiencies were identified.

DATA FOR ENTRY INTO ISIS

Special Study

PC code	MRID #	Study type	Species	Duration	Route	Dosing method .	Dose range (ppm)	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
128008	45404902	special study	rat	2 weeks	oral	food	15,000	~1500	NA	NA	liver	centrilobular hypertrophy and CYP450 induction

NA = Not applicable