DATA EVALUATION RECORD

BAS 510 F

STUDY TYPE: HORMONE AND ENZYME INDUCTION IN WISTAR RATS

MRID 45404903

7/23/2002

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 (96.3%)

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

CITATION: Mellert, W., Deckardt, K., Leibold, E., et. al. (2001). BAS 510 F - Hormone and enzyme induction study in Wistar rats, administration in the diet for 4 weeks. Experimental Toxicology and Ecology of BASF Aktiengesellschaft, D-67056 Ludwigshafen/Rhein, Germany. BASF Reg. Doc. No. 2001/1000141, February 28, 2001. MRID 45404903. Unpublished.

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

EXECUTIVE SUMMARY: In a study done to investigate the effects on thyroid homeostasis and hepatic microsomal glucuronyltransferases (MRID 45404903), BAS 510 F (96.3% a.i., Lot no. 97/179-4 N46) 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 ~1000 mg/kg bw/day) for four weeks. Blood was collected periodically during the study to determine triiodothyronine (T_3), thyroxine (T_4), and thyroid stimulating hormone (TSH) concentrations. At the end of the study, the rats were killed, the livers removed, homogenized, and microsomes prepared to determine glucuronyltransferase activity.

Treatment with 15,000 ppm BAS 510 F for 28 days induced a slight (statistically significant at some intervals) decrease in circulating T_3 and T_4 levels in male rats apparent by the 4th day and through the end of the study. No similar effect was noted in female rats. In addition, the test material induced an increase in circulating TSH levels in treated male and female rats within two days that persisted through the remainder of the study. The activities of all three liver microsomal glucuronyltransferases investigated (*p*-nitrophenol, 4-methylumbeliferone, and 4-hydroxybiphenyl) were increased with treatment. The induction of these enzymes has been shown to increase the metabolism of thyroid hormones resulting in constant thyroid stimulation by TSH. This in turn has been associated as one of the factors in the development of thyroid neoplasia.

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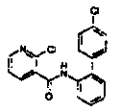
The study results suggest that BAS 510 F results in disruption of thyroid homeostasis by decreasing circulating T_3 and T_4 levels and increasing TSH. This is likely the result of hepatic microsomal glucuronyltransferase induction . The study is considered **acceptable/nonguideline.**

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 #:	97/179-4 N46
Purity:	96.3%
Compound Stability:	Proven stable by reanalysis
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:	2-3 months; males, 395-452 g; females, 238-258 g.	
Source:	Boehringer Ingelheim Pharma KG, Biberach/Riss, Germany	
Housing:	Singly in type DK III stainless steel wire mesh cages	
Diet:	9433 LL Meal, Eberle Nafag AG, Gossau, 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:	20 days	

B. STUDY DESIGN:

1. In life dates:

Start: July 3, 2000; End: August 1-2, 2000

2. Animal assignment:

Animals were assigned by weight using computer randomization to the test groups noted in Table 1.

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TABLE 1. Study Design					
Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg bw/day)		Number of animals	
		Male	Female	Male	Female
0	0	0	0	5	5
1	15,000	957	1197	5	5

3. **Dose selection rationale**

The dose was the highest dose used in previous studies.

4. **Diet preparation and analysis**

An appropriate amount of the test material was weighed and mixed throughly with a small amount of diet. The premix was added to an appropriate amount of diet and mixed in a mixer for 10 minutes to create the 15,000 ppm diet; the only concentration used in the study. The test diet was prepared only once during the study.

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 (97.5-100% of initial content).

Concentration analysis: Test material diet concentration was within 2.2% of nominal.

For the purposes of this study, the diet preparation was acceptable.

5. **Statistics:** Body weights, body weight change, food consumption and food efficiency were compared by the Welch t-test. Thyroid hormone concentrations of treated versus control animals were done by the non-parametric Mann-Whitney U-test (two-sided). Comparisons of liver weight and glucuronyltransferase activities of treated versus control animals were done by the Wilcoxon test (one-sided). 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:** Clinical examinations were conducted daily.

2. **Body weight:** Body weights were obtained prior to test article administration to randomize animals and weekly during the 28-day study.

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

4. **Food efficiency:** This was calculated on individual body weights and food consumption.

5. **Drinking water consumption:** Observed daily by visual inspection to detect overt changes in volume.

6. **Blood collection:** Before the start of the study (day -3) and on days 2, 4, 7, 14, 21, and 28, blood was collected (non-fasted animals, without anesthesia) from the retroorbital venous plexus to determine the serum concentrations of triiodothyronine (T_3), thyroxine (T_4), and thyroid stimulating hormone (TSH).

7. **Sacrifice:** After 29-30 days of treatment, the rats were sacrificed by decapitation under CO_2 anesthesia and the livers removed and weighed. The livers were then homogenized and microsomes prepared using standard preparation techniques.

8. **Clinical bioanalytics:** Rats were anesthetized with carbon dioxide and decapitated. Livers were removed and weighed. Liver microsomes were prepared according to Standard Operating Procedures.

   Hormone Analyses: The analyses of T_3, T_4, and TSH were done by competitive radioimmunoassay (RIA)

   Total protein: The method for the determination of microsomal protein was not reported but is assumed to be by the biuret method.

   p-Nitrophenol-glucuronyltransferase activity (pNP-GT): pNP-GT activity was measured spectrophotometrically at 405 nm according to the method of Bock (Bock et al., 1983, Biochem. Pharmacol. 32, 953-955).

   4-Methylumbeliferone-glucuronyltransferase (MUF-GT): MUF-GT activity was measured fluorimetrically at an excitation wavelength of 315 nm and an emission wavelength of 365 nm according to the method of Lilienblum (Lilienblum et al., 1982. Biochem. Pharmacol. 31, 907-913).

   4-Hydroxybiphenyl-glucuronyltransferase (HOBI-GT): HOBI-GT activity was measured fluorimetrically at an excitation wavelength of 278 nm and an emission wavelength of 327 nm according to the method of Lilienblum (Lilienblum et al., 1982. Biochem. Pharmacol. 31, 907-913).

II. RESULTS

A. OBSERVATIONS:

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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.

Table 2. Body weights ± S.D. (g) and body weight gains ± S.D. (g) for male and female rats treated with BAS 510 F for 29-30 days

Day	Dose (ppm)			
	Males		Females	
	0	15000	0	15000
0 body weight	424 ± 18	439 ± 9	245 ± 7	250 ± 6
28 body weight	459 ± 18	477 ± 11	257 ± 8	265 ± 6
0-28 body weight gain	35 ± 25a	38 ± 8	11 ± 4	15 ± 4

Data extracted from Report pages 38-41.
5/sex/group
a = individual values were (g): 70, 41, 30, 29 and 2.

C. FOOD CONSUMPTION AND COMPOUND INTAKE: Neither food consumption nor efficiency appeared to have been affected by test article administration. 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 25% in males and 22% in females after four-weeks of treatment (Table 2). Likewise, the liver to body weight ratios for male and female rats were increased 20% and 18%, respectively.

TABLE 3. Absolute liver weight (g) and liver to body weight (%) from male and female rats treated with BAS 510 F for 29-30 days [a]

Dose (ppm)	Day 28 body weight		Absolute Liver Weight (g)		Liver to Body Wt. (%) [b]	
	Male	Female	Males	Females	Males	Females
0	459±18	257±8	18.1 ± 1.76	9.6 ± 0.69	3.94 ± 0.33	3.74 ± 0.27
15,000	477±11 (101)	265±6 (101)	22.6* ± 1.11 (125)	11.7* ± 0.53 (122)	4.74** ± 0.23 (120)	4.42*** ± 0.22 (118)

Derived from data on pp 66, 68 & 75 of MRID 45404903
[a] n =5 for all groups
[b] Statistical analyses calculated by reviewer using one-way t-test with equal variances
* = p≤0.05; ** = p≤0.02; *** = p≤0.002
parentheses are percents of control value

2. Hormone concentrations:

T_3 - A persistent decrease in circulating T_3 to as much as ~70% of control was found in treated male rats throughout the 28 day study, although the decrease was statistically

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significant only on day 4 (Table 4). There was a sporadic decrease of T_3 in female rats, however, no steady decrease in circulating levels was found.

T_4 - As with T_3, a persistent decrease in circulating T_4 was found in treated male rats throughout the 28-day study (Table 4). The decreases were statistically significant by the 4th day of treatment to the end of the study. No similar effect was found in female rats.

TSH - The circulating TSH concentration of both male and female rats was increased within two days of treatment and was consistently elevated throughout the study (relatively large range of individual values within all groups at all intervals).

TABLE 4. Thyroid hormone time-dependent concentrations in rats treated with BAS 510 F for 28 days

Group/Day	-3	2	4	7	14	21	28
T_3 (nmol/L) - Males							
Control	1.3 ± 0.20	1.4 ± 0.21	1.38 ± 0.26	1.44 ± 0.33	1.44 ± 0.24	1.05 ± 0.20	1.13 ± 0.26
15,000 ppm	1.12 ± 0.14	1.18 ± 0.20	0.96* ± 0.17	1.13 ± 0.3	1.11 ± 0.27	0.74 ± 0.21	0.79 ± 0.24
% of control	86	84	69	79	77	70	71
T_3 (nmol/L) - Females							
Control	1.57 ± 0.14	1.67 ± 0.32	1.63 ± 0.28	1.64 ± 0.26	1.62 ± 0.20	1.14 ± 0.29	1.15 ± 0.26
15,000 ppm	1.65 ± 0.11	1.67 ± 0.12	1.35 ± 0.11	1.26** ± 0.08	1.55 ± 0.28	1.13 ± 0.10	1.06 ± 0.13
% of control	105	101	83	76	96	99	92
T_4 (nmol/L) - Males							
Control	64.2 ± 7.2	66.2 ± 5.8	63.4 ± 4.7	67.5 ± 7.5	66.0 ± 7.0	73.0 ± 6.0	72.7 ± 8.2
15,000 ppm	62.0 ± 3.7	57.7 ± 6.1	51.0** ± 6.8	50.5** ± 2.0	48.3** ± 4.6	56.5** ± 4.6	59.9** ± 4.2
% of control	97	87	81	75	73	77	82
T_4 (nmol/L) - Females							
Control	50.5 ± 7.3	46.5 ± 8.8	48.9 ± 8.2	51.8 ± 8.9	51.8 ± 11.2	55.7 ± 11.7	51.9 ± 9.4
15,000 ppm	56.2 ± 5.3	55.2 ± 1.9	49.8 ± 6.4	45.1 ± 7.5	48.5 ± 6.7	51.5 ± 6.4	50.2 ± 11.1
% of control	111	119	102	87	94	93	97
TSH (ng/mL) - Males							
Control	19.38 ± 9.15	22.21 ± 9.64	20.59 ± 11.6	20.12 ± 12.83	19.32 ± 4.89	14.50 ± 4.40	23.17 ± 8.83
15,000 ppm	19.28 ± 6.06	28.63 ± 12.05	26.20 ± 12.09	32.76 ± 9.98	36.53** ± 13.8	41.02** ± 11.5	38.88* ± 14.4
% of control	100	129	127	163	189	283	168
TSH (ng/mL) - Females							
Control	9.70 ± 4.03	9.31 ± 1.70	9.15 ± 2.09	12.00 ± 7.35	11.70 ± 2.34	9.09 ± 2.87	10.28 ± 1.59
15,000 ppm	10.77 ± 3.94	16.77* ± 8.39	18.21** ± 6.3	24.80 ± 8.79	32.39** ± 8.89	19.27** ± 5.53	22.32** ± 5.45
% of control	111	180	199	207	277	212	217

Data from pp. 46-51 of MRID 45404903
* = $p \leq 0.05$; ** = $p \leq 0.02$
n=5 for all groups

3. Microsomal glucuronyltransferase activities:

pNP-GT - As shown in Table 5, pNP-GT activity was increased ~2-fold in treated male rats and 1.25-fold in treated female rats relative to their respective control rats.

MUF-GT - As shown in Table 5, MUF-GT activity was increased 2-fold in treated male rats and 2.4-fold in treated female rats relative to their respective controls.

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HOBI-GT - HOBI-GT activity was increased ~3-fold in treated male and female rats relative to control rats (Table 5).

TABLE 5. Microsomal glucuronyltransferase activities in male and female rats treated with BAS 510 F for 28 days.

Group	pNP-GT (nmol/min/mg prot.)		MUF-GT (FU/min/mg prot.)		HOBI-GT (FU/min/mg prot.)	
	Male	Female	Male	Female	Male	Female
Control	1.47 ± 0.36	5.27 ± 0.32	894 ± 180	462 ± 133	56.7 ± 10.4	45.6 ± 25.8
15.000 ppm	2.84* ± 0.48 (193)a	6.59* ± 0.29 (125)	1789* ± 333 (200)	1111* ± 204 (240)	175.2* ± 39.8 (309)	131.8* ± 23.3 (289)

Data from pp. 53-55 MRID 45404903
* = $p \leq 0.05$
n=5 for all groups
a = percent of control

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: According to the study authors, treatment of male and female Wistar rats with 15,000 ppm BAS 510 F for four weeks decreased circulating T_3 and T_4 concentrations and increased TSH. They concluded that the metabolism of T_4 by hepatic glucuronide conjugation appeared to be responsible for the increased levels of TSH and that these effects were apparent within two days of treatment with the test material.

B. REVIEWER COMMENTS: In this study, feeding 15,000 ppm BAS 510 F for 28 days induced a slight (statistically significant at some intervals) decrease in the circulating levels of T_3 and T_4 in male rats apparent from the 4th day of treatment through the end of the study. No similar effect was noted in the concentration of thyroid hormones of treated female rats. Excluding other factors, the decrease in these thyroid hormone concentrations would be of little biological concern as they were relatively minor, likely within the laboratory's normal ranges, and not the magnitude associated with hypothyroid states.

Treatment with the test material also induced an increase in circulating TSH levels in treated male and female rats within two days that persisted, and became larger in males, through the remainder of the study. As with the thyroid hormone concentrations, the increase in TSH of male and female rats may have been within the established normal range for the laboratory. When all thyroid function data are viewed, however, there appears to be a response in the thyroid-pituitary loop indicative of thyroid stimulation. The constant stimulation is likely the result of the hepatic microsomal glucuronyltransferases which were shown to be induced by treatment (p-nitrophenol, 4-methylumbeliferone and 4-hydroxybiphenyl). The induction of these enzymes has been shown to result in a thyroid hormone imbalance that results in thyroid follicular hyperplasia and hypertrophy. This condition is associated with increased thyroid tumor formation.

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

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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	45404903	special study	rat	4 weeks	oral	food	15,000	~1000	NA	NA	thyroid, liver	↑ glucuronyl-transferase activity, ↑ TSH, ↓ T3 & T4

NA = Not applicable