DATA EVALUATION RECORD

BAS 510 F

Study Type: Non-Guideline 4-Week Reversibility Study in Rats

Work Assignment No. 4-01-181 (MRID 45550601)

7/24/2002

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Non-guideline 4-Week Reversibility Study in Rats (feeding)

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

TEST MATERIAL (PURITY): BAS 510 F (94.1% a.i.)

SYNONYMS: 2-chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide

CITATION: Mellert, W., K. Deckardt, et. al. (2001) BAS 510 F- Reversibility Study in Wistar Rats, Administration in the Diet for 4 Weeks Followed by Recovery Periods of 4 Weeks and 3 Months. Experimental Toxicology and Ecology, BASF, Ludwigshafen/Rhein, Germany. Laboratory Project Id.: 37C0179/97176, November 26, 2001. MRID 45550601. Unpublished.

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

EXECUTIVE SUMMARY: In a non-guideline 4-week reversibility toxicity study (MRID 45550601), BAS 510 F (94.1% a.i.; Batch no. N46) was administered to 15 male Wistar (CrlGlxBrlHan:Wi) rats/dose in the diet. The dose levels were 0, 100, 2500, or 15000 ppm (equivalent to 0, 7.7, 190.3, and 1137.4 mg/kg/day). There were 3 subgroups at each dose consisting of 5 rats which were allowed no recovery, 4 weeks recovery, or 13 weeks recovery. This study was performed to determine the reversibility of substance-induced effects on the thyroid and liver. There were no compound related effects on mortality, clinical signs, body weight, food or water consumption, food efficiency, or either total triiodothyronine or thyroxine serum levels. No adverse effect was observed at 100 ppm.

After 4 weeks of dosing, treatment-related increases ($p<=0.02$) over control values in thyroid stimulating hormone (TSH) were observed at 2500 (incr 68%) and 15000 ppm (incr 87%). No increases in TSH were observed at these doses after 4 or 13 weeks recovery. Additionally, absolute thyroid weights were increased ($p<=0.01$) in the 2500 and 15000 ppm groups at the end of dosing (incr 47-49%), and remained increased ($p<=0.05$, 15000 ppm group no statistical significance, with a 4 week recovery period) through the 4 and 13 week recovery periods (incr 20-33%). Relative (to body) thyroid weights were dose-dependently increased in all the treated

2

groups after dosing (incr 17-50%) and remained increased in the 2500 and 15000 ppm groups after 4 weeks recovery (incr 20%, each), and after 13 weeks recovery (not statistically significant). In addition, hypertrophy of thyroid follicular epithelial cells and diffuse follicular hyperplasia were observed at 2500 (minimal to moderate, 3-4/5 treated vs 0/5 controls) and 15000 ppm (slight to severe, 5/5 treated vs 0/5 controls, each) after dosing. After a 4 or 13 week recovery period, the incidence and severity of these microscopic abnormalities did not exceed the concurrent controls.

After 4 weeks of dosing, absolute and relative liver weights were increased ($p<=0.05$) at 2500 (incr 22-25%) and 15000 (incr 45-48%) ppm. The liver was enlarged in all 15000 ppm males (vs 0 controls). Centrilobular hypertrophy and liver portal (zone 1) fatty changes were observed at 2500 (minimal to slight, 4/5 treated vs 0/5 controls, each) and 15000 ppm (minimal to moderate; 5/5 treated, each). After a 4 or 13 week recovery period, these liver abnormalities were not observed.

The Sponsor stated that it was shown that BAS 510 F induces the liver microsomal enzyme system in rats. The Sponsor proposed that this induction results in increased glucuronidation of thyroxine, resulting in an increase in TSH secretion as a compensatory response of the physiological negative feedback system. Increased TSH results in the increased thyroid weight. The reviewers agree that this is a plausible explanation for the effects observed in the liver and thyroid, as well as the reversibility of these conditions.

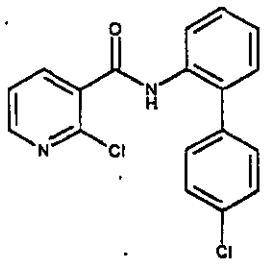
The submitted study is classified as **acceptable/non-guideline**. The stated purpose of determining the reversibility of substance-induced effects on the thyroid and liver was fulfilled.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** BAS 510 F
   Description: White solid
   Lot/Batch #: N46
   Purity: 94.1% a.i.
   Stability of compound: Stable in the diet for up to 32 days at room temperature.
   CAS #: 188425-85-6
   Structure:



2. **Vehicle:** Diet

3. **Test animals:**
   Species: Rats
   Strain: Wistar (CrlGlxBrlHan:Wi)
   Age and weight at the start of dosing: 56±2 days old; 208.0-245.6 g males
   Source: Charles River, Sulzfeld, Germany
   Housing: Individually in type DK III stainless steel wire mesh cages
   Diet: Ground Kliba maintenance diet rat/mouse/hamster meal (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*
   Water: Tap water, *ad libitum* (water bottles)
   Environmental conditions:
   Temperature: 20-24°C
   Humidity: 30-70%
   Air changes: Not reported
   Photoperiod: 12 hours light/12 hours dark
   Acclimation period: 7 days

B. STUDY DESIGN: This study was performed to determine the reversibility of substance-induced effects on the thyroid and liver.

1. **In life dates** - Start: 3/27/01 End: 7/30/01

2. **Animal assignment** - The animals were randomly assigned (stratified by weight) to the test groups shown in Table 1.

Table 1. Study design [a]

Nominal Dose (ppm)	Actual Dose (mg/kg/day)	# of Assigned Animals		
		Subgroup A, No Recovery	Subgroup B, 4 Week Recovery	Subgroup C, 13 Week Recovery
0	0	5	5	5
100	7.7	5	5	5
2500	190.3	5	5	5
15000	1137.4	5	5	5

a Data obtained from the study report, page 14. All animals were treated for 4 weeks prior to the recovery period. Only males were treated.

3. **Dose-selection rationale** - The Sponsor stated that the dose-selection was based on the results of previous studies; however, the methodology and results of these studies were not provided.

4. **Treatment preparation and analysis** - Dietary formulations were prepared once, before study initiation, by diluting a concentrated test material-feed mixture (premix) with appropriate amounts of Ground Kliba maintenance diet rat/mouse/ hamster meal to achieve the desired test concentrations. Dietary formulation analyses were performed prior to study initiation. Stability of BAS 510 F in the diet was assessed in the 100 ppm diet mix for up to 32 days at room temperature. Homogeneity was assessed in the 100 and 15,000 ppm dietary formulations. The concentration of each dietary formulation was also measured.

Results - Homogeneity Analysis: Range of % coefficient of variation at both doses, 1.8-2.6

Stability Analysis: % of Day 0: 97.9

Concentration Analysis: % of nominal:

100 ppm	96.0±2.5
2500 ppm	94.1
15000 ppm	94.3±1.7

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

5. Statistics

Parameter	Statistical procedure
Food consumption, body weight, body weight change, and food efficiency	Two-sided analysis of variance (ANOVA) followed by Dunnett's test when $p \leq 0.05$
Thyroid hormone parameters, and liver and thyroid organ weights	Two-sided Kruskal-Wallis followed by the Wilcoxon test when $p \leq 0.05$

C. METHODS

1. Observations - The animals were monitored for moribundity and mortality twice daily Monday through Friday and once each Saturday, Sunday, and holidays. Clinical examinations were conducted daily.

2. Body weight - Each animal was weighed prior to treatment, weekly throughout the study beginning on Day 0, and at necropsy. Body weight gain (g) was also reported for weekly intervals throughout the study.

3. Food/water consumption/efficiency and compound intake - Food consumption was recorded weekly and reported as mean g/animal/day. Food efficiency (g body weight gain/g food consumed X 100) was reported for each study week. Compound intake values (mg/kg/day) were calculated using the individual consumption and body weight data and the nominal dose. Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume.

4. Thyroid hormone levels - Blood was collected from the retroorbital venous plexus without anesthesia in the morning of Days -1, 28, 56, and 119 from all surviving non-fasted animals. The concentrations of total triiodothyronine (T_3), total thyroxine (T_4), and thyroid stimulating hormone (TSH) were determined by radioimmunoassay.

5. Sacrifice and pathology - All animals were fasted for 16-20 hours, sacrificed by decapitation under CO_2 anesthesia, and subjected to gross pathological examination. The liver and thyroid (with parathyroids) were weighed and examined histologically.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs of toxicity** - No clinical observations were attributed to treatment. Incidental alopecia was observed in one control and one 2500 ppm male.

2. **Mortality** - All animals survived to the scheduled necropsy.

B. BODY WEIGHT AND WEIGHT GAIN: No treatment-related effect was observed on body weights (Table 2). Subgroups A, B and C (5 animals/dose/group) were terminated at the end of dosing, after a 4-week recovery, and after a 13-week recovery, respectively.

Table 2. Body weights ± S.D. (g) and body weight gains ± S.D. (g) in male rats fed BAS 510 F

Day	Dose (ppm)			
	0	100	2500	15000
4 weeks of dosing				
0 body weight	228 ± 10	224 ± 8	225 ± 9	221 ± 8
28 body weight	326 ± 24	320 ± 11	333 ± 20	322 ± 23
0-28 body weight gain	98 ± 16	96 ± 11	108 ± 14	101 ± 17
4 weeks of dosing + 4 weeks of recovery				
0 body weight	229 ± 11	226 ± 7	229 ± 13	226 ± 11
56 body weight	392 ± 35	380 ± 33	383 ± 30	376 ± 31
0-56 body weight gain	163 ± 26	154 ± 27	154 ± 20	150 ± 23
4 weeks of dosing + 13 weeks of recovery				
0 body weight	225 ± 8	229 ± 10	227 ± 12	228 ± 11
119 body weight	420 ± 32	443 ± 49	450 ± 25	400 ± 16
0-119 body wt gain	195 ± 30	215 ± 41	224 ± 17	172 ± 18

Data extracted from Report pages 51-58.
5 males/group

C. FOOD CONSUMPTION, COMPOUND INTAKE AND FOOD UTILIZATION

1. **Food consumption** - No treatment-related effects were observed on food or water consumption.

2. **Food efficiency** - No treatment-related effect was observed on food efficiency.

3. **Compound intake** - The mean achieved dosages are reported in Table 1.

D. THYROID HORMONE LEVELS: After 4 weeks of dosing, treatment-related increases (percent of control) ($p \leq 0.02$) in thyroid stimulating hormone (TSH) were observed at 2500 (168%) and 15000 ppm (187%). No increases in TSH were observed at these doses after 4 or 13 weeks recovery. No treatment-related effects were observed on total T_3 or T_4.

Table 3. Mean thyroid stimulating hormone levels (µg/l) found in male rats fed BAS 510 F. [a]

Dose (ppm)	Before treatment (n-=15)	After 4 weeks of treatment (n=15)	After 4 weeks of treatment and 4 weeks of recovery (n=10)	After 4 weeks of treatment and 13 weeks of recovery (n=5)
0	7.84	9.72	8.59	7.21
100	7.24 (92)	9.83 (101)	7.09 (83)	7.57 (105)
2500	7.79 (92)	16.36** (168)	6.60 (77)	7.19 (100)
15000	7.88 (101)	18.20*** (187)	7.90 (92)	6.68 (93)

a These data were obtained from the in-text table on page 32 of this study. Numbers listed parenthetically represent the reported percent difference from controls.
** Significantly different from controls at $p \leq 0.02$.
*** Significantly different from controls at $p \leq 0.002$.

E. SACRIFICE AND PATHOLOGY

1. **Organ weight** - Terminal body weights were similar to controls (Table 4). After 4 weeks of dosing, absolute and relative liver weights were increased ($p \leq 0.05$) at 2500 (122-125% of control) and 15000 (145-148%) ppm, but were not increased following a 4 or 13 week recovery period. Absolute thyroid weights were increased ($p \leq 0.01$) in the 2500 and 15000 ppm groups at the end of dosing (147-149%), and remained increased ($p \leq 0.05$, except in the 15000 ppm group with a 4 week recovery period) through the 4 and 13 week recovery periods (120-133%). Relative (to body) thyroid weights were dose-dependently increased in all the treated groups after dosing (117-150%) and remained increased (statistically significant) in the 2500 and 15000 ppm groups after the 4 week recovery period (120%, each), as well as after the 13 week recovery (120%, not statistically significant) (see Table 5).

8

Table 4. Mean (±SD) absolute (g) and relative to body (%) liver and thyroid weights and terminal body weights (g) of male rats fed BAS 510 F. [a]

Dose (ppm)	After 4 weeks of treatment	After 4 weeks of treatment and 4 weeks of recovery	After 4 weeks of treatment and 13 weeks of recovery
Absolute Liver			
0	7.710±0.8	9.138±0.927	8.756±1.106
100	7.894±0.367 z9102)	8.278±0.685 (91)	9.62±0.991 (110)
2500	9.628±0.97* (125)	8.888±0.537 (97)	9.258±0.612 (106)
15,000	11.16±1.065** (145)	8.698±0.956(95)	8.396±0.532 (96)
Absolute Thyroid			
0	16.6±1.517	17.0±0.707	17.8±2.864
100	19.0±1.581 (114)	17.6±1.949 (104)	23.2±2.49* (130)
2500	24.8±2.864** (149)	20.4±2.191** (120)	23.4±2.793* (131)
15,000	24.4±5.32** (147)	20.6±3.286 (121)	23.6±2.966* (133)
Terminal Body Weight			
0	291.84±20.573	351.74±31.748	389.76±34.682
100	286.92±12.218 (98)	341.88±28.314 (97)	418.42±52.777 (107)
2500	299.52±14.571 (103)	343.42±24.064 (98)	418.64±22.758 (107)
15,000	285.8±19.335 (98)	337.84±28.662 (96)	371.98±14.943 (95)
Relative Liver			
0	2.639±0.15	2.603±0.225	2.248±0.218
100	2.754±0.142 (104)	2.424±0.128 (93)	2.309±0.192 (103)
2500	3.214±0.268* (122)	2.59±0.069 (100)	2.211±0.075 (98)
15,000	3.901±0.191** (148)	2.574±0.160 (99)	2.256±0.064 (100)
Relative Thyroid			
0	0.006±0.001	0.005±0.000	0.005±0.001
100	0.007±0.0** (117)	0.005±0.001 (100)	0.006±0.001 (120)
2500	0.008±0.001** (133)	0.006±0.001* (120)	0.006±0.000 (120)
15,000	0.009±0.002** (150)	0.006±0.001* (120)	0.006±0.001 (120)

a These data were obtained from the tables on pages 75-80 of this study. Numbers listed parenthetically represent the reported percent difference from controls (page 33), n=5.

* Significantly different from controls at $p \leq 0.05$.
** Significantly different from controls at $p \leq 0.01$.

Table 5. Individual relative thyroid weights (% of body weights) in male rats fed BAS 510F.

	Dose (ppm)			
	0	100	2500	15000
after 4 weeks of dosing	6,6,6,5,5a	7,7,7,6,6	9,8,8,8,7	11,10,8,8.6
after 4 weeks of recovery	5,5,5,5,4	6,6,5,5,4	7,6,6,6,5	7.6,6.6,5
after 13 weeks of recovery	6,5,5,4,4	6,6,6,5,5	6,6,6,5,5	7,7,6,6,6

Data extracted from Report pages 131-142.
a = values are as 0.006% of body weight.

2. **Gross pathology** - The liver was enlarged in all 15000 ppm rats after 4 weeks of treatment (vs 0 controls), but were not enlarged after either of the recovery periods. Erosion/ulcers in the glandular stomach, enlarged iliac lymph nodes, and single occurrences of various abnormalities were observed, but were not dose-dependent.

3. **Microscopic pathology** - After 4 weeks of dosing, centrilobular hypertrophy and liver portal (zone 1) fatty changes were observed in the 2500 (minimal to slight, 4/5 treated vs 0/5 controls, each) and 15000 ppm (slight to moderate, 5/5 treated) groups (Table 6a). Hypertrophy of follicular epithelial cells and diffuse follicular hyperplasia in the thyroid were also observed at 2500 (slight or minimal to moderate, 3-4/5 treated vs 0/5 controls) and 15000 ppm (slight to severe, 5/5 treated) groups (Table 6b). After a 4 or 13 week recovery period, treatment-related liver and thyroid abnormalities were not observed.

Table 6a. Selected hepatic microscopic lesions (# affected/5) found in male rats fed BAS 510 F for 4 weeks and no recovery period. [a]

Hepatic lesion	0 ppm	100 ppm	2500 ppm	15000 ppm
Centrilobular hypertrophy				
minimal	0	0	2	0
slight	0	0	2	4
moderate	0	0	0	1
Liver portal (zone 1) fatty change				
minimal	0	0	3	1
slight	0	0	1	2
moderate	0	0	0	2

a These data were obtained from the table on page 84 of this study. These microscopic lesions were not observed following a 4- or 13-week recovery period.

10

Table 6b. Selected thyroid microscopic lesions (# affected/5) found in male rats fed BAS 510 F for 4 weeks and no recovery period or with a 4 or 13 week recovery period. [a]

Thyroid lesion	0 ppm	100 ppm	2500 ppm	15000 ppm
No recovery period				
Hypertrophy of follicular epithelial cells				
slight	0	0	2	1
moderate	0	0	2	2
severe	0	0	0	2
Diffuse follicular hyperplasia				
minimal	0	0	1	0
slight	0	0	1	3
moderate	0	0	1	2
4 week recovery period				
Hypertrophy of follicular epithelial cells				
minimal	0	1	0	1
slight	2	0	1	1
Diffuse follicular hyperplasia				
minimal	2	0	0	0
13 week recovery period				
Hypertrophy of follicular epithelial cells				
minimal	1	1	1	0
Diffuse follicular hyperplasia				
minimal	1	0	0	0

a These data were obtained from the tables on pages 84-86 of this study.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS: No clinical signs of toxicity were observed. At 2500 and 15000 ppm, TSH levels were increased following 4 weeks of treatment but returned to normal during the 4 week recovery period. Reversible effects were also observed in the liver (increased organ weight, gross enlargement, and centrilobular hypertrophy) and thyroid (increased organ weight, and follicular cell hypertrophy and hyperplasia).

B. REVIEWER'S COMMENTS: There were no compound related effects on mortality, clinical signs, body weight, food or water consumption, food efficiency, or total T_3 or T_4 serum levels. No adverse effect was observed at 100 ppm.

After 4 weeks of dosing, absolute and relative liver weights were increased ($p \leq 0.05$) at 2500 (122-125% of control) and 15000 (145-148%) ppm. The liver was enlarged in all 15000 ppm males (vs 0 controls). Centrilobular hypertrophy and liver portal (zone 1) fatty changes were observed at 2500 (minimal to slight, 4/5 treated vs 0/5 controls, each) and 15000 ppm (minimal to moderate, 5/5 treated, each). After a 4 or 13 week recovery period, these liver abnormalities were not observed.

After 4 weeks of dosing, treatment-related increases ($p \leq 0.02$) in TSH were observed at 2500 (168% of control) and 15000 (187%) ppm. No increases in TSH were observed at these doses after 4 or 13 weeks recovery. Additionally, absolute thyroid weights were increased ($p \leq 0.01$) in the 2500 and 15000 ppm groups after dosing (147-149%), and remained increased ($p \leq 0.05$, except in the 15000 ppm group with a 4 week recovery period) through the 4 and 13 week recovery periods (120-133%). Relative (to body) weights were increased dose-dependently in all the treated groups after dosing (117-150%) and remained increased in the 2500 and 15000 ppm groups after 4 weeks of recovery (120%, each), as well as after 13 weeks recovery (table of individual values). In addition, hypertrophy of thyroid follicular epithelial cells and diffuse follicular hyperplasia were observed at 2500 (minimal to moderate, 3-4/5 treated vs 0/5 controls, each) and 15000 ppm (slight to severe, 5/5 treated vs 0/5 controls, each) after dosing. After a 4 or 13 week recovery period, the incidence and severity of these microscopic abnormalities did not exceed the concurrent controls.

The Sponsor stated that it was shown that BAS 510 F induces the liver microsomal enzyme system in rats. The Sponsor proposed that this induction results in increased glucuronidation of T_4, resulting in an increase in TSH secretion as a compensatory response of the physiological negative feedback system. An increased TSH level results in increased thyroid weight. The reviewers agree that this is a plausible explanation for the effects observed in the liver and thyroid, as well as the reversibility of these conditions.

The submitted study is classified as **acceptable/non-guideline**. The stated purpose of determining the reversibility of substance-induced effects on the thyroid and liver was fulfilled.

12

DATA FOR ENTRY INTO ISIS

Non-guideline 4-Week Reversibility Study - rats

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	45550601	Non-guideline *reversibility*	Rats	4 week with up to 13 weeks of recovery	Oral	Diet	7.7-1137.4	0, 7.7, 190.3, 1137.4			Liver, Thyroid	