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TOXIC SUBSTANCES

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MEMORANDUM

DATE: November 14, 2002

SUBJECT: BAS 510 F: Report of the Cancer Assessment Review Committee

FROM: Jessica Kidwell, Executive Secretary Jessica Kidwell
Cancer Assessment Review Committee
Health Effects Division (HED) (7509C)

TO: Alan Levy, Toxicologist
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Health Effects Division (HED) (7509C)

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Registration Division (7505C)

The Cancer Assessment Review Committee met on September 25, 2002 to evaluate the carcinogenic potential of BAS 510 F. Attached please find the Final Cancer Assessment Document.

cc: R. Hill
J. Pletcher
Y. Woo
Tom Morris/Katherine Adcock, Health Canada's Pesticide Management Regulatory Agency (PMRA)

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CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

BAS 510 F

PC Code 128008

FINAL REPORT

November 14, 2002

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

BAS 510 F CANCER ASSESSMENT DOCUMENT FINAL

DATA PRESENTATION: Alan C. Levy
Alan Levy, Toxicologist

DOCUMENT PREPARATION: Jessica Kidwell
Jessica Kidwell, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam

Karl Baetcke

Marion Copley

Nancy McCarroll

Esther Rinde

Joycelyn Stewart

Clark Swentzel

Linda Taylor

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

John Pletcher, Consulting Pathologist see attached sheet

Lori Brunsman, Statistical Analysis

OTHER ATTENDEES: William Drew (HED), Steve Knizner (HED), Maria Rodriguez (RD), Tom Morris and Katherine Adcock, Health Canada's Pesticide Management Regulatory Agency (PMRA) (teleconference)

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EXECUTIVE SUMMARY

On September 25, 2002, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of BAS 510 F.

Dr. Alan Levy of Registration Action Branch 2 presented the chronic toxicity study and the carcinogenicity study in Wistar rats as well as the carcinogenicity study in C57BL/6 J Rj mice by: describing the experimental design; reporting on survival and body weight effects, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, the adequacy of the dose levels tested; and presenting the weight of the evidence for the carcinogenicity of BAS 510 F. Dr. Levy also discussed the toxicology, metabolism and mutagenicity studies as well as structure-activity relationships and mechanistic data.

BAS 510 F was administered in the diet: to 20 Wistar rats/sex/dose at concentrations of 0, 100, 500, 2500 or 15000 ppm for 24 months in a chronic toxicity study; to 50 Wistar rats/sex/dose at concentrations of 0, 100, 500, 2500, or 15000 ppm for 24 months in a carcinogenicity study [Due to excessive body weight losses, both 15000 ppm groups were sacrificed after 17 months and not further analyzed]; and to 50 C57BL/6 J Rj mice/sex/dose at concentrations of 0, 80, 400, 2000 or 8000 ppm for 18 months.

The CARC concluded that BAS 510 F showed evidence of carcinogenicity based on the following:

- When the data were combined from the rat chronic toxicity study and the carcinogenicity study, male rats had a significant increasing trend ($p<0.01$), and significant differences in the pair-wise comparison of the 2500 ppm dose group with the controls for thyroid follicular cell adenomas ($p<0.05$). There was no treatment-related increase in thyroid follicular cell carcinomas. However, when the one follicular cell carcinoma from the control was included, there was only a significant increasing trend for combined adenomas and carcinomas ($p<0.05$) in males. The increased incidence of the thyroid follicular cell adenomas exceeded the historical control mean and range. The CARC considered the increase in thyroid follicular cell adenomas to be treatment-related in males. This was supported by thyroid hypertrophy and hyperplasia of follicular cells at 2500 ppm, increased thyroid weights, and mechanistic data.

- When the data were combined from the rat chronic toxicity study and the carcinogenicity study, female rats had a significant increasing trend ($p<0.05$) for thyroid follicular cell adenomas. The CARC considered the increasing trend for adenomas to be treatment-related in females. This tumor response was consistent with the male response in terms of tumor types and hyperplasia. No carcinomas were observed.

- The highest dose tested of 2500 ppm was considered to be adequate in both sexes to assess the carcinogenicity of BAS 510 F in Wistar rats. This was based on changes in clinical chemistry/enzymes and organ weights, as well as liver and thyroid lesions.

- There was no treatment-related increase in any tumors in male or female mice.

- The highest dose tested of 8000 ppm (1345 mg/kg/day in males; 1804 mg/kg/day in females) is above the limit dose for both sexes, and, therefore, was considered to be adequate, but not excessive, to assess the carcinogenicity of BAS 510 F in C57BL/6 J Rj mice.

- BAS 510 F was not mutagenic in a battery of acceptable *in vitro* and *in vivo* studies.

- No appropriate structural analogues were located for comparison purposes.

- The mode of action data were not adequate to characterize the dose-response in relationship to tumor formation. The mechanistic data were useful, however, in the overall weight-of-the-evidence in corroborating the tumor and non-tumor data.

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July 1999), the CARC classified BAS 510 F into the category **"Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential"**, and, therefore, the quantification of human cancer risk is not recommended.

I. INTRODUCTION

Data submitted by the Registrant was reviewed jointly as a work share product under the North American Free Trade Agreement's Technical Working Group on Pesticides (NAFTA TWG) Joint Review Program by HEALTH Canada's Pest Management Regulatory Agency (PMRA) and the U.S. EPA. Review of the toxicology data on BAS 510 F has been completed by the U.S. EPA and has been submitted to PMRA for comment and/or concurrence.

On September 25, 2002, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of BAS 510 F.

II. BACKGROUND

BAS 510 F, (3-pyridinecarboxamide,2-chloro-N-(4'chloro(1,1'-biphenyl)-2-yl) is a fungicide. BAS 510 F Turf Fungicide is a 70% WG formulation of the BAS 510 F active ingredient and is intended for use on golf course turf only. BAS 510 02 F Crop Fungicide is a 70% WG and includes the terrestrial food group uses of berries crop group, bulb vegetable crop group, beans, canola, carrots, fruiting vegetable crop group, grapes, lettuce, peanuts, potatoes, strawberries, stone fruit, tree nut crop group and pistachio. The BAS 516 02 F Crop Fungicide is a 38% WG mixture of BAS 510 F and pyraclostrobin. The target uses of BAS 516 02 F are berries crop group, bulb vegetables crop group, carrots, grapes, strawberries, stonefruit, tree nut crop group and pistachio.

Tolerances are requested for the target crops and crop groups of berries crop group, bulb vegetable crop group, beans, canola, carrots, fruiting vegetable crop group, grapes, lettuce, peanuts, tuberous and corm vegetables, strawberries, stone fruit, tree nut crop group and pistachio. The associated livestock tolerances for milk, muscle, fat and meat-by-products are also requested. A waiver is requested for tolerances in poultry.

Tolerances are also requested for crops to be used in rotation with the target crops: root vegetables crop group; leaves of root vegetable crop group: leafy vegetables crop group: brassica leafy vegetables crop group; legume vegetables crop group; foliage of legume vegetable crop group; cucurbit crop group; cereal grains crop group; forage, fodder and straw of cereal grains crop group; grass forage and fodder and hay crop group, non-grass animal feed crop group, soybean seed, forage, hay and aspirated grain fractions, flax seed, sunflower seed, cotton seed and gin by-products and mint.

III. EVALUATION OF CARCINOGENICITY EVIDENCE

1. Chronic Toxicity Study in Wistar Rats

Reference: W. Mellert, K. Deckardt, W. Kaufmann, *et al* (2001) Chronic toxicity study in Wistar rats; Administration in the diet for 24 months. Experimental Toxicology and Ecology BASF Aktiengesellschaft, D-67056 Ludwigshafen, Rhein, Germany. Laboratory Project No. 82C0179/97091, BASF Registration Document Number 2001/1000114, February 28, 2001. MRID 45404827. Unpublished.

A. Experimental Design

In a chronic toxicity study (MRID 45404827) BAS 510 F (94.4% a.i., lot no. N37, Tox-batch III) was administered to 20 Wistar rats/sex/dose in the diet at concentrations of 0, 100, 500, 2500 or 15000 ppm (equivalent to 0, 4.4, 21.9, 110.0 or 739.0 mg/kg bw/day for males and 0, 5.9, 30.0, 150.3 or 1000.4 mg/kg bw/day for females, respectively) for 24 months. Due to excessive body weight losses, both 15000 ppm groups were sacrificed after 17 months and not further analyzed.

B. Discussion of Tumor Data

Survival Analysis

The statistical evaluation of mortality in this study indicated no significant incremental changes with increasing doses of BAS 510 F in male rats (Memo, L. Brunsman, 8/16/02, TXR No. 0050919). In female rats, there was a statistically significant decreasing trend for mortality, as well as a statistically significant pair-wise comparison of the high dose with the control. For females, the largest number of dead occurred in the control and 500 ppm groups (7/20) compared with one death in the 2500 ppm group (HDT).

Tumor Analysis: Chronic Toxicity Study

There were no statistically significant trends or pair-wise comparisons of the dosed groups with the controls in either male or female rats regarding thyroid follicular cell adenomas (Memo, L. Brunsman, 8/16/02, TXR No. 0050919). No carcinomas were observed. The statistical analyses of both sexes were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. See Tables 1 and 2 for chronic toxicity tumor analysis results.

Regarding thyroid follicular cell adenoma, historical control tumor data provided by the Registrant showed that the mean rate of adenoma (male/female) was 1.0/0.8% from 35/41 studies (32/37 feeding studies) that included 1470/1710 animals, the range was 0-6/0-10%, and ≥ 6% adenoma occurred in 1/ 2 of the 35/41 studies.

Table 1. BAS 510 F - Wistar Chbb: THOM (SPF) Chronic Toxicity Rat Study

Male Thyroid Tumor Rates+ and Exact Trend Test
And Fisher's Exact Test Results (p-values)

	Dose (ppm)			
	0	100	500	2500
C-Cell Adenomas# (%)	4/20 (20)	2/20 (10)	3[a]/20 (15)	4/20 (20)
p =	0.3274	0.3307	0.5000	0.6526
Follicular Cell Adenomas# (%)	0/20 (0)	0/20 (0)	2[b]/20 (10)	1/20 (5)
p =	0.1989	1.0000	0.4949	0.5000

+ Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.

a First c-cell adenoma observed at week 79, dose 500 ppm

b First follicular cell adenoma observed at week 105, dose 500 ppm.

No c-cell or follicular cell carcinomas were observed.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. BAS 510 F - Wistar Chbb: THOM (SPF) Chronic Toxicity Rat Study

Female Thyroid Tumor Rates+ and Exact Trend Test and Fisher's Exact Test Results (p-values)

	Dose (ppm)			
	0	100	500	2500
C-Cell Adenomas # (%)	3/20 (15)	5[a]/20 (25)	7/20 (35)	4/20 (20)
p =	0.4434	0.3474	0.1367	0.5000
Follicular Cell Adenomas # %	0/20 (0)	0/20 (0)	1[b]/20 (5)	0/20 (0)
p =	0.7500	1.0000	0.5000	1.0000

+ Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.
a First c-cell adenoma observed at week 102, dose 100 ppm.
b First follicular cell adenoma observed at week 106, dose 500 ppm.
No c-cell or follicular cell carcinomas were observed.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-neoplastic Lesions

An increased incidence of liver centrilobular hypertrophy occurred at 2500 ppm ($p \leq 0.01$) in both sexes, and liver eosinophilic foci were increased ($p \leq 0.05$) in 2500 ppm males. The hypertrophy was characterized by an increase in the hepatocyte size and organelle content, and the eosinophilic foci had cytoplasmic inclusions.

Incidences of thyroid follicular cell diffuse hypertrophy and focal hyperplasia were increased in both sexes at 2500 ppm , but were not statistically significant. The liver and thyroid lesions correlated with the minor increases in the weights of these two organs. Several lesions of unknown etiology and toxicological significance were found as well, including an increased incidence of ovarian cysts ($p \leq 0.05$) in 500 and 2500 ppm females, and rectal lumen parasites in 2500 ppm males.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

Adequacy of dosing for both the chronic toxicity study and the carcinogenicity study is discussed under Section 3 (Chronic Toxicity Study and Carcinogenicity Study in Rats Combined)/Subsection B (Adequacy of Dosing).

2. Carcinogenicity Study in Wistar Rats

Reference: W. Mellert, K. Deckardt, W. Kaufmann, *et al.* (2001) Carcinogenicity study in Wistar rats; Administration in the diet for 24 months. Experimental Toxicology and Ecology BASF Aktiengesellschaft, D-67056 Ludwigshafen/Rhein, Germany. Laboratory Project No. 82C0179/97090, BASF Registration Document Number 2001/1000115, February 28, 2001. MRID 45404828. Unpublished.

A. Experimental Design

In a carcinogenicity study (MRID 45404828) BAS 510F (94.4% a.i., lot no. N37, Tox-batch III) was administered to 50 Wistar rats/sex/dose in the diet at concentrations of 0, 100, 500, 2500 or 15000 ppm (equivalent to 0, 4.6, 23.0, 116.1 and 768.8 mg/kg bw/day for males and 0, 6.0, 29.7, 155.6 and 1024.4 mg/kg bw/day for females) for 24 months. The investigators determined that 15000 ppm was causing excessive weight loss in females and mortality in males and these groups were sacrificed after approximately 17 months and not further analyzed.

B. Discussion of Tumor Data

Survival Analysis

The statistical evaluation of mortality in this study indicated no significant incremental changes with increasing doses of BAS 510 F in either male or female rats (Memo, L. Brunsman, 8/16/02, TXR No. 0050919).

Tumor Analysis: Carcinogenicity Study

Male rats had significant increasing trends in thyroid follicular cell adenomas at $p<0.01$, and combined adenomas and/or carcinomas at $p<0.05$. There were no significant differences in the pair-wise comparisons of the dosed groups with the controls. There was 1/50 males with carcinoma in the control group (none in any of the other groups).

Female rats had a significant increasing trend in thyroid follicular cell adenomas at $p<0.05$. There were no significant differences in the pair-wise comparisons of the dose groups with the

controls. No carcinomas were observed.

The statistical analyses of both sexes were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons (Memo, L. Brunsman, 8/16/02, TXR No. 0050919). See Tables 3 and 4.

Regarding thyroid follicular cell adenoma, historical control tumor data provided by the Registrant, showed that the mean rate of adenoma (male/female) was1.0/0.8% from 35/41 studies (32/37 feeding studies) that included1470/1710 animals, the range was 0-6/0-10%, and ≥ 6% adenoma occurred at 1/ 2 of the 35/41 studies.

Table 3. BAS 510 F - Wistar Chbb: THOM (SPF) Carcinogenicity Rat Study

Male Thyroid Follicular Cell tumor Rates[+] and Exact trend Test
And Fisher's Exact Test results (p-values)

	Dose (ppm)			
	0	100	500	2500
Adenomas (%)	0/50 (0)	0/50 (0)	1/50 (2)	4a/50 (8)
p =	0.0054**	1.0000	0.5000	0.0587
Carcinomas (%)	1b/50 (2)	0/50 (0)	0/50 (0)	0/50 (0)
p =	0.2500	0.5000	0.5000	0.5000
Combined (%)	1/50 (2)	0/50 (0)	1/50 (2)	4/50 (8)
p =	0.02145*	0.5000	0.7525	0.1811

+ Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.
a First adenoma observed at week 87, dose 2500 ppm.
b First carcinoma observed at week 107, dose 0 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. BAS 510 F - Wistar chbb:THOM (SPF) Carcinogenicity Rat Study

Female Thyroid Follicular Cell Tumor Rates+ and Exact Trend Test
And Fisher's Exact Test Results (p-values)

	Dose (ppm)			
	0	100	500	2500
Adenomas# (%)	0/50 (0)	1a/50 (2)	0/49 (0)	3/50 (6)
p =	0.0342*	0.5000	1.0000	0.1212

+ Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.
a First adenoma observed at week 106, dose 100 ppm.
No carcinomas were observed.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-neoplastic Lesions

The incidences of notable non-neoplastic lesions are presented in Table 5. The incidence of liver centrilobular hypertrophy was increased significantly at 2500 ppm ($p \leq 0.01$) in males and females. The incidence of liver eosinophilic foci was increased slightly, but not statistically, in 500 and 2500 ppm males, but was not seen in females. The hypertrophy was characterized by an increase in the hepatocyte size and organelle content, and a ground-glass appearance sometimes containing eosinophilic droplets. The eosinophilic foci in 2500 ppm males had cytoplasmic inclusions.

The incidences of thyroid follicular cell diffuse hypertrophy and of focal hyperplasia were also increased in both sexes of 2500 ppm rats, but the change was significant ($p \leq 0.01$) only in males. The thyroid lesions were correlated with an increase in the weight of this organ in males. The decreased incidence ($p \leq 0.05$) of focal degeneration of the adrenal cortex in 2500 ppm females was likely incidental to treatment, and the small (non-significant) increase in the incidence of urinary bladder diffuse papillary hyperplasia in males was of unknown etiology.

Table 5. Incidence of non-neoplastic microscopic pathology findings in rats fed BAS 510 F for 2 years

Organ: lesion	Dietary concentration (ppm)							
	0	100	500	2500	0	100	500	2500
	Males				Females			
Liver: Centrilob. hypertrophy	0/50	0/50	2/50	27/50**	0/50	0/50	0/50	11/50**
Eosinophilic foci	3/50	4/50	8/50	9/50	0/50	0/50	0/50	0/50
Thyroid glands:								
Hypertr. Follic. Cell. diffuse	2/50	5/50	6/50	22/50**	2/50	0/50	0/50	4/50
Hyperpl. Follic. Cell. focal	1/50	1/50	1/50	9/50**	2/50	2/50	1/50	7/50

Data from pp. 41-42 and 145-208, MRID 45404828.
*p ≤ 0.05. **p ≤ 0.01: Significantly different from controls, determined by reviewer using Fisher Exact Test.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

Adequacy of Dosing for both the chronic toxicity study and the carcinogenicity study is discussed under Section 3 (Chronic Toxicity Study and Carcinogenicity Study in Rats Combined)/Subsection B (Adequacy of Dosing).

3. Chronic Toxicity Study and Carcinogenicity Study in Rats Combined

A. Discussion of Tumor Data

Survival Analysis

With data from the two 2-year rat studies combined, mortality statistical analyses did not show any adverse effects regarding pair-wise comparison or trend for males or females (Memo, L. Brunsman, 8/16/02, TXR No. 0050919).

Tumor Analysis: Carcinogenicity and Chronic Toxicity Studies Combined

Male rats had a significant increasing trend ($p<0.01$), and significant differences in the pair-wise comparison of the 2500 ppm dose group with the controls for thyroid follicular cell adenomas ($p<0.05$). For carcinomas in males, there was no statistical significance for either pair-wise comparison or trend. However, when the one follicular cell carcinoma from the control was included, there was only a significant increasing trend ($p<0.05$) for combined adenomas and carcinomas in males.

Female rats had a significant increasing trend for thyroid follicular cell adenomas at $p<0.05$. There were no significant differences in the pair-wise comparisons of the dosed groups with the

controls. No carcinomas were observed.

The statistical analyses of both sexes were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. See Tables 6 and 7 for carcinogenicity and chronic toxicity studies combined tumor analysis results.

Regarding thyroid follicular cell adenoma, historical control tumor data provided by the Registrant showed that the mean rate of adenoma (male/female) was 1.0/0.8% from 35/41 studies (32/37 feeding studies) that included 1470/1710 animals, the range was 0-6/0-10%, and ≥ 6% adenoma occurred in 1/2 of the 35/41 studies.

Table 6. BAS 510 F - Wistar Chbb: THOM (SPF) Carcinogenicity and Chronic Toxicity Combined Rat Studies

Male Thyroid Follicular Cell Tumor Rates+ and Exact Trend Test And Fisher's Exact Test Results (p-values)

	Dose (ppm)			
	0	100	500	2500
Adenomas (%)	0/70 (0)	0/70 (0)	3/70 (4)	5[a]/70 (7)
p =	0.0045**	1.0000	0.1223	0.0290*
Carcinomas (%)	1[b]/70 (1)	0/70 (0)	0/70 (0)	0/70 (0)
p =	0.2500	0.5000	0.5000	0.5000
Combined (%)	1/70 (1)	0/70 (0)	3/70 (4)	5/70 (7)
p=	0.0130*	0.5000	0.3098	0.1043

+ Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.

[a] First adenoma observed at wk 87, dose 2500 ppm.

[b] First carcinoma observed at week 107, dose 0 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 7. BAS 510 F - Wistar Chbb: THOM (SPF) Carcinogenicity and Chronic Toxicity Combined Rat Studies

Female Thyroid Follicular Cell Tumor rates+ and Exact Trend Test and Fisher's Exact Test Results (p-values)

Dose (ppm)				
	0	100	500	2500
Adenomas# %	0/70 (0)	1[a]/70 (1)	1[a]/69 (1)	3/70 (4)
p =	0.0439*	0.5000	0.4964	0.1223

+ Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.
[a]First adenomas observed concurrently at week 106 at doses 100 and 500 ppm.
No carcinomas were observed.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

B. Adequacy of Dosing

Chronic Toxicity

The highest dose tested was 2500 ppm, which was equivalent in mg/kg/day to 110.0 in males and 150.3 in females. The study started with an additional dose of 15000 ppm (equivalent to 739.0/1000.4 mg/kg/day for males/females), but both sexes at this dose were terminated after approximately 17 months because the Registrant felt that there were, " ...severe effects on body weight which were expected to progress with time ..." (The terminated rats were not further analyzed). The early termination of this higher-dose group is questionable. It is felt that the 17-month terminated animals should at least have been subjected to histopathological examination (for tumor incidence).

Carcinogenicity Study

The highest dose tested was 2500 ppm which was equivalent in mg/kg/day to 116.1 in males and 155.6 in females. The study started with an additional dose of 15000 ppm (equivalent to 768.8/1024.4 mg/kg/day for males/females), but both sexes at this dose were terminated after approximately 17 months because the Registrant felt that, " ... there had been a progressive increase in mortality in the 15000 ppm males ..." and "... body weight in the high dose females

started to decrease after about 6 months of treatment. This decline became more rapid after about 14 months ..." The early termination of this higher-dose group is questionable. It is felt that the 17-month terminated animals should at least have been subjected to histopathological examination (for tumor incidence).

Combined Chronic Toxicity and Carcinogenicity Study

Because the original high-dose rats in both 2-year studies (15000 ppm) were terminated after about 17 months, there was a question as to the adequacy of the 2-year dose of 2500 ppm. The CARC concluded that the dosing at 2500 ppm was adequate for both the chronic and carcinogenicity rat studies (See Table 8). At the highest dose tested for 24 months (2500 ppm), the following effects were noted in males: an increase in serum gamma glutamyl transferase (SGGT) of 1-18 times over control, an increase in absolute and/or relative thyroid weights, an increase in the incidences of liver eosinic foci and centrilobular hypertrophy, an increase in the incidences of thyroid follicular cell hypertrophy and thyroid follicular cell hyperplasia, and an increase in the incidence of thyroid adenomas.

For females (highest dose tested for 24 months was 2500 ppm), the following effects were noted at this dose: SGGT was elevated 2-3 times over control, an increase in the incidence of liver centrilobular hypertrophy, a slight increase in the incidence of thyroid follicular cell hypertrophy and/or hyperplasia, and an increase in the incidence of thyroid adenomas.

The above effects were all observed at 2500 ppm in both studies with the next lowest dose of 500 ppm not showing any of these observations.

In the 90-day rat study, males given 137 mg/kg/day showed increases in absolute and relative thyroid weights and increased incidence of thyroid hyperplasia as well as follicular epithelial hypertrophy. Females at 395 mg/kg/day had increases in absolute and relative thyroid weights.

Table 8. BAS 510 F: Non-neoplastic Effects in Chronic Toxicity, Carcinogenicity, and 90-day Studies

parameter	dose (ppm)	2-year chronic toxicity		2-year carcinogenicity		90-day toxicity	
		male	female	male	female	male	female
body weight and body weight gain	100	↔	↔	↔	↔	↔	↔
	500	↔	↔	↔	↔	↔	↔
	2000	NE	NE	NE	NE	↔	↔
	2500	↔	↔	↔	↓6-24%	NE	NE
	5000	NE	NE	NE	NE	↔	↔
	15000	↓8-11%	↓17-21%	↔	↓4-21%	↔	↔
enzyme sggt	100	↔	↔	|	|	↔	↔
	500	↑1-3x	↔	|	|	↔	↔
	2000	NE	NE	NE	NE	↑	↔
	2500	↑1-18x	↑2-3x	|	|	NE	NE
	5000	NE	NE	|	|	↑	↑
	15000	↑4-21x	↑4-≥10x	|	|	↑	↑
liver weights	100	↔	↔	↔	↔	↔	↔
	500	↔	↔	↔	↔	↔	↔
	2000	NE	NE	NE	NE	↔	↔
	2500	↔	↑rel	↔	↔	NE	NE
	5000	NE	NE	NE	NE	↑rel	↑ab/rel
	15000	NE	NE	NE	NE	↑ab/rel	↑ab/rel
thyroid weights	100	↔	↔	↔	↔	↔	↔
	500	↔	↔	↔	↔	↔	↔
	2000	NE	NE	NE	NE	↑ab/rel	↔
	2500	↑ab	↔	↑ab/rel	↔	NE	NE
	5000	NE	NE	NE	NE	↔	↑ab/rel
	15000	NE	NE	NE	NE	↑ab/rel	↑ab/rel
histopath non-neoplastic liver	100	↔	↔	↔	↔	↔	↔
	500	↔	↔	↔	↔	↔	↔
	2000	NE	NE	NE	NE	↔	↔
	2500	↑ef/ch	↔ef, ↑ch	↑ef/ch	↑ch	NE	NE
	5000	NE	NE	NE	NE	↑ch	↔
	15000	NE	NE	NE	NE	↑ch	↑ch
histopath non-neoplastic thyroid	100	↔	↔	↔	↔	↔	↔
	500	↔	↔	sl↑htr	↔	↔	↔
	2000	NE	NE	NE	NE	↑htr/hyp	↔
	2500	sl↑htr/hyp	sl↑htr	↑htr/hyp	sl↑htr/hyp	NE	NE
	5000	NE	NE	NE	NE	↑htr/hyp	↔
	15000	NE	NE	NE	NE	↑htr/hyp	↔
histopath thyroid adenomas	0	0	0	0	0	0	0
	100	0	0	0	1	all	all
	500	2	1	1	0	doses	doses
	2500	1	0	4	3		

↔ = no difference from control ↑ = increase from control ↓ = decrease from control
NE = no dose or not examined ab = absolute rel = relative sl = slight
ef = eosinophilic foci ch = centrilobular hypertrophy htr = hypertrophy thyroid follicular cell
hyp = hyperplasia thyroid follicular cell

4. Carcinogenicity Study in C57BL/6 J Rj Mice

Reference: W. Mellert, K. Deckardt, K. Kuttler, *et al.* (2001) BAS 510 F, Carcinogenicity study in C57BL mice; Administration in the diet for 18 months. Experimental Toxicology and Ecology BASF Aktiengesellschaft, D-67056 Ludwigshafen/Rhein, Germany. Laboratory Project No. 76 C0179/97103, BASF Registration Document Number 2001/1000116, February 28, 2001. MRID 45404901. Unpublished.

A. Experimental Design

In a carcinogenicity study (MRID 45404901) BAS 510 F (94.4% a.i., batch no. N37, Tox-batch III) was administered to 50 C57BL/6 J Rj) mice/sex/dose in the diet at concentrations of 0, 80, 400, 2000 or 8000 ppm (equivalent to 0, 13, 65, 331 and 1345 mg/kg bw/day for males and 0, 18, 90, 443 and 1804 mg/kg bw/day for females) for 18 months.

B. Discussion of Tumor Data

Neither sex had statistically significant increases in the incidence or total number of any type of tumor (primary, benign or malignant), or in the number of tumor-bearing animals.

D. Non-neoplastic Lesions

The liver was affected by treatment in both sexes. The incidence of liver peripheral (periportal) hypertrophy was increased significantly ($p \leq 0.01$) at 8000 ppm in both sexes and in 2000 ppm females. The hypertrophy was minimal or slight (severity grade 1 or 2), and the cytoplasm of the enlarged cells stained eosinophilic and appeared "delicate granular." Females given $\leq$ 400 ppm had a shift from diffuse to centrilobular hepatocyte fatty infiltration (comparable severity grades), which was correlated at $\geq$ 2000 ppm with peripheral hypertrophy. An increased incidence of oval cell proliferation (minimal or slight in most animals) was also seen in 8000 ppm females ($p \leq 0.05$), although there was an inconsistent dose-response from 80-2000 ppm. These liver effects are correlated with the increases in liver weights in both sexes, and are consistent with an adaptive response of the liver to a xenobiotic toxicant.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

The highest dose tested was 8000 ppm which was equivalent in mg/kg/day to 1345 in males and 1804 in females. These are above limit dose. It is therefore considered that the dose for assessment of carcinogenic potential in mice was adequate.

IV. TOXICOLOGY DATA

1. Metabolism

Two rat studies were conducted: metabolism (MRID 45404918) and pharmacokinetic (MRID 45404919). The studies used non-labelled, diphenyl label and pyridine label BAS 510 F. Dosing was as follows: diphenyl = single dose of 50 mg/kg label, single dose of 500 mg/kg label and 14 doses of unlabelled plus one dose of labelled; pyridine = single dose of 500 mg/kg labelled.

BAS 510 F was readily absorbed and excreted following a single oral 50 mg/kg dose. At a single 500 mg/kg or 15 doses of 500 mg/kg, absorption was saturated. Excretion was mainly by feces (80-98%). Biliary excretion was 40-50% of fecal activity at 50 mg/kg and 10% at 500 mg/kg. Absorption was about 56% at 50 mg/kg and 13-17% at 500 mg/kg. Excretory patterns were similar by gender or radiolabel position. Metabolites (hydroxylation and conjugation products) were consistent with Phase I oxidation reactions followed by Phase II conjugation with glucuronic acid or sulfate, or by conjugation of the parent with glutathione with cleavage to sulfate metabolites.

2. Mutagenicity

There are five acceptable mutagenicity studies on technical grade BAS 510 F. Together, they satisfy the revised mutagenicity guideline of 1991 (OPP Pesticide Assessment Guidelines. Subdivision F, Series 84, Addendum 9) which are applicable to all new active ingredients. Results in all five studies were negative for mutagenic potential. The following summarize these studies:

A. *Salmonella/Escherichia*/Mammalian Activation Gene Mutation (Engelhardt, G., 1998, MRID 45404913)

EXECUTIVE SUMMARY: In repeat reverse gene mutation assays in bacteria (MRID 45404913), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2(uvrA) of *E. coli* were exposed to BAS 510 F (95.3% a.i., Batch No.: N 26) in DMSO, initially at concentrations of 22, 110, 550, 2750 or 5500 µg/plate, with and with mammalian metabolic activation (S9-mix), using a standard plate assay (EXPERIMENT 1). The same five strains were exposed to the test material in a repeat assay at concentrations of 20, 100, 500, 2500 or 5000 µg/plate in the presence and absence of S9-mix using a preincubation assay (EXPERIMENT 2). The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

BAS 510 F was tested up to and beyond a limit concentration of 5000 µg/plate. Precipitation was seen at concentrations of 500 µg/plate and higher and weak cytotoxicity was evident at

concentrations of 2500 µg/plate and higher in the *S. typhimurium* strains. There was no increase in the number of revertants per plate over the solvent control value in any bacterial strain at any BAS 510 F concentration in either assay, with or without S9-mix. The solvent and positive control values were appropriate for the respective strains and within the testing laboratory's historical control ranges.. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/Guideline**. It satisfies the Test Guideline OPPTS 870.5100[1]; OECD 471 requirements for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

B. Mammalian Cells in Culture Gene Mutation Assay in Chinese Hamster CHO Cells (Engelhardt, G., 2000, MRID 45404914)

EXECUTIVE SUMMARY: In repeat mammalian cell gene mutation assays at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus (MRID 45404914), Chinese hamster CHO-K1 cells cultured *in vitro* were exposed initially for four hours to BAS 510 F (94.4% a.i., Batch No.: N 37) in DMSO at concentrations of 15.625, 31.25, 62.5, 125, 250 or 500 µg/mL in the presence and absence of mammalian metabolic activation (S9-mix). Subsequently, a second experiment was conducted using test material concentrations of 10.24, 25.6, 64, 160, 400 or 1000 µg/mL with and without S9-mix with a repeat of the nonactivated test at concentrations of 3.125, 6.25, 12.5, 25, 50 or 100 µg/mL. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

BAS 510 F was tested up to cytotoxic and precipitating concentrations. In the preliminary cytotoxicity assay without S9-mix, the relative cloning efficiency was reduced to approximately 10% at test material concentrations of 500 µg/mL and higher and a precipitate was seen in the culture medium at concentrations of 50 µg/mL and higher. In the presence of S9-mix the relative cloning efficiency was below 10% at concentrations of 500 µg/mL and higher. Results of the mutation assays were not affected by pH or osmolality changes. The mutant frequency of all BAS 510 F treated cultures was within the testing laboratory's historical solvent control ranges in both assays, with and without S9-mix. Excessive cytotoxicity occurred in the absence of S9-mix in the second mutation assay necessitating a repeat at lower doses. The solvent and positive controls induced the appropriate responses within the testing laboratory's historical control ranges. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/Guideline.** It satisfies the guideline requirement for Test Guideline OPPTS 870.5300, OECD 476 for *in vitro* mutagenicity (mammalian forward gene mutation) data.

C. *In vitro* Chromosomal Aberration (Engelhardt, G., 1999. MRID 45404915)

EXECUTIVE SUMMARY: In repeat mammalian cell cytogenetics assays (chromosomal aberrations) (MRID 45404915), Chinese hamster V79 cell cultures were exposed to BAS 510 F (94.4% a.i., batch # N 37) in DMSO in two independent assays. In the first assay, cells were exposed to the test material for four hours at concentrations of 0, 20.0, 100.0 or 500.0 µg/mL with and without metabolic activation and the cells harvested 14 hours after termination of treatment. In the second cytogenetic assay, cells were exposed to the test material for 18 hours at concentrations of 0, 31.25, 62.5 or 125.0 µg/mL in the absence of S9-mix and harvested immediately after exposure. Cells were also exposed to 0 or 125 µg/mL of test material for 28 hours in the absence of S9-mix and harvested immediately after exposure. Cells were exposed to test material concentrations of 0, 125.0, 250.0 or 500.0 µg/mL for four hours in the presence of S9-mix and harvested 24 hours after termination of exposure.

BAS 510 F was tested up to precipitating concentrations. A preliminary cytotoxicity test at concentrations up to 3500 µg/mL showed precipitation at BAS 510 F concentrations of 100 µg/mL and higher. Little or no cytotoxicity, as evaluated by mitotic index and cell morphology changes, was seen with or without S9-mix. The test material did not change the pH or osmolality of the treatment medium. Both structural and numerical aberrations were recorded. There were no statistically significant increases in the percentage of metaphases with structural aberrations or numerical aberrations, either including or excluding gaps, at any test material concentration or exposure/harvest time scenario. The types and frequencies of aberrations in test material treated cells were similar to those of the solvent controls. The solvent and positive controls induced the appropriate responses within the testing laboratory's historical control ranges. **There was no evidence of chromosomal aberration induction over background.**

This study is classified as **Acceptable/Guideline**. It satisfies the guideline requirement for an *in vitro* mammalian cytogenetics (chromosomal aberrations) assay in Chinese hamster V79 cells; OPPTS 870.5375; OECD 473.

D. *In vivo* Mammalian Cytogenetics - Micronucleus Assay in Mouse Bone Marrow Cells (Engelhardt, G., 1999, MRID 45404916)

EXECUTIVE SUMMARY: In a mouse bone marrow micronucleus assay (MRID 45404916), 5 NMRI male mice/dose were treated via two intraperitoneal injections 24 hours apart with BAS 510 F (94.4% a.i., batch # N 37) at doses of 0, 500, 1000 or 2000 mg/kg bw. Bone marrow cells were harvested 24 hours after the second treatment. The vehicle was 0.5% carboxymethyl cellulose.

There were signs of toxicity during the study. A preliminary toxicity test with two i.p. injections of 2000 mg/kg test material in male and female mice showed squatting posture,

piloerection and poor general state but no mortality. No sex differences were seen, therefore, males only were used in the micronucleus assay. In the micronucleus assay, the same clinical signs seen in the preliminary toxicity test were seen during the first four hours following both the first and second injections. All mice in the low dose group appeared normal 24 hours after each injection while all mice in the mid- and high-dose groups showed squatting posture at this time after the first injection and piloerection at this time after the second injection. The frequencies of micronucleated PCEs in the 500, 1000 and 2000 mg/kg groups were 1.4‰, 1.3‰ and 1.2‰, respectively, compared to the solvent control value of 1.2‰. There were no statistically significant differences between groups. Likewise, there were no statistically significant differences between groups in the frequency of micronucleated NCEs. Of the micronuclei that were observed in PCEs, virtually all were small micronuclei. The PCE/NCE ratios indicated no bone marrow cytotoxicity. BAS 510 F was tested to a toxic limit dose. The solvent and positive controls (cyclophosphamide and vincristine sulfate) induced the appropriate responses within the testing laboratory's historical control ranges.. **There was no statistically significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any dose.**
This study is classified as **Acceptable/Guideline**. It satisfies the guideline requirement for Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

E. Other Mutagenicity: Unscheduled DNA Synthesis in Primary Rat Hepatocytes/Mammalian Cell Cultures, (Engelhardt, G., 2000, MRID 45404917

EXECUTIVE SUMMARY: In repeat unscheduled DNA synthesis assays (MRID 45404917), primary rat hepatocyte cultures were exposed to BAS 510 F (94.4% a.i., Batch # N 37) in DMSO at concentrations of 0, 5, 10, 50, 100, 250, 500, 750, and 1000 µg/mL for 18 - 20 hours in the first UDS experiment. Due to excess cytotoxicity, the first experiment was repeated at BAS 510 F concentrations of 0, 0.5, 1.0, 5.0, 10.0, 50.0, 100.0, 250.0, and 500.0 µg/mL for 18 - 20 hours. A second experiment was conducted at BAS 51 F concentrations of 1.563, 3.125, 6.250, 12.500, 25.000, and 50.000 µg/mL for 18 - 20 hours.

BAS 510 F was tested up to cytotoxic concentrations. An upper dose of 500 µg/mL was chosen for the first UDS experiment based on results of a preliminary cytotoxicity test using lactate dehydrogenase activity and lactate concentration as the measure of cytotoxicity. A precipitate was seen in the culture medium at test material concentrations of 50 µg/mL and higher. The test material did not change the pH or osmolality of the culture medium. Cytotoxicity was greater than expected in the initial experiment and the cells were not evaluated for UDS. In the repeat first experiment, BAS 510 F concentrations of 1, 5, 10 and 50 µg/mL were evaluated for UDS. Concentrations of 100 - 500 µg/mL were excessively cytotoxic and were not evaluated. There was no evidence of induced UDS (all net nuclear grain counts were well below zero) or any increase in the number of cells in repair (net nuclear grain counts ≥ 5) compared to the solvent control in the first experiment. BAS 510 F concentrations of 6.25, 12.50, 25.00 and 50.00 µg/mL were evaluated in the second UDS

experiment. The results confirmed those of the first experiment, with no induction of UDS (all net nuclear grain counts were well below zero) or increases in the percentage of cells in repair. The mean net nuclear grain counts were -5.74 ± 4.44 and 29.01 ± 15.00 for the solvent and positive controls, respectively, in the first experiment. Comparable values in the second experiment were -4.40 ± 3.62 and 10.24 ± 10.45 for the solvent and positive controls, respectively. The control values were within the testing laboratory's historical control ranges. **There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts] was induced.**

This study is classified as **Acceptable/Guideline.** It satisfies the guideline requirement for Test Guideline OPPTS 870.5550; OECD 482/486 for other mutagenicity data.

3. Structure-Activity Relationships

HED is not currently aware of any analog for this chemical.

4. Subchronic and Chronic Toxicity

In subchronic and chronic oral toxicity studies in rats, mice and dogs, there was no evidence suggesting cumulative toxicity. The thyroid and liver appeared to have been the only affected organs. In the 2-year rat (two studies) and 90-day rat studies, there were increases in thyroid weights and/or microscopic changes (follicular cell hyperplasia and hypertrophy). In both of the 2-year studies, the NOAELs for thyroid changes were 21.9 or 23.0 mg/kg/day for males and 30.0 or 29.7 mg/kg/day for females. The LOAELs in these studies were 110.0 and 116.1 mg/kg/day for males and 150.3 and 155.6 mg/kg/day for females. For the 90-day study, the NOAELs for males and females were 34 and 159 mg/kg/day with LOAELs being 137 and 395 mg/kg/day.

There were no thyroid effects observed in the 18-month mouse study (> 1000 mg/kg/day) or the 12-month dog study (HDT 544-593 mg/kg/day). The 2-generation reproduction study in rats did not show any effects on the thyroid at doses >1000 mg/kg/day.

No effects on the liver were reported in either of the 2-year rat studies or in the 18-month mouse study. The 12-month dog study showed elevated alkaline phosphatase activities (both sexes) and hepatic weights (males only). In this study, the NOAELs were 21.8/22.1 mg/kg/day for males/females with the LOAELs being 57.4/58.3 mg/kg/day for males/females. For the 90-day mouse study, there were increased liver weights and an increased incidence of marked fatty change in the liver in males only (NOAELs = 197/2209 mg/kg/day for males/females; LOAELs = 788/>2209 mg/kg/day for males/females). In the 2-generation reproduction rat study, both generation males (not females) showed hepatocyte degeneration (NOAEL = 101.2-123.9 mg/kg/day; LOAEL = 1034.5-1295.4 mg/kg/day).

5. Mode of Action Studies

A. Hepatic Enzyme Induction Study in Rats (MRID 45404902)

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 15000 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 cytochrome 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 15000 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 or female rats. However, the *O*-dealkylation of pentaoxyresorufin by CYP450 2B4 was approximately double in 15000 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 15000 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.

B. Hormone and Enzyme Induction Study in Rats (MRID 45404903)

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 15000 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 15000 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.

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.**

C. Reversibility Study: 4-Week Administration Followed by 4 Weeks Recovery or 13 Weeks Recovery (MRID 45550601)

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 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.

D. Discussion of Liver and Thyroid Parameters

LIVER:

In the rat, absolute and relative liver weights were statistically increased (over control value) in most studies at doses ≥ 1000 mg/kg in males and/or females. This was also seen in the mouse studies at ≥ 300 mg/kg and in dog studies ≥ 500 mg/kg.

Alkaline phosphatase as well as alanine and aspartate aminotransferases were measured in some rat studies. There were either little or no increases compared with control values. Of the three enzymes, only alkaline phosphatase activity was increased in dogs, and that occurred ≥ 57 mg/kg in males and/or females. In 90-day and 18-month mouse studies (where measured) there were little or no changes from control values.

Hyperplasia was not observed in any of the studies. Hypertrophy incidences were increased at ≥110 mg/kg in rat studies, at ≥ 330 mg/kg only in the 18-month mouse

study and was not observed in either the 90-day or 12-month dog studies.

THYROID:

In the rat, absolute and relative thyroid weights were statistically increased (over control value) in all studies at doses ≥110 mg/kg in males and/or females. No effects were noted in the mouse studies. In dogs, an increase only in absolute weights was observed at doses ≥57 mg/kg.

Incidences of hyperplasia and hypertrophy were increased at ≥ 110 mg/kg in rat studies. No differences were noted between the incidences of these parameters compared with controls in either mice or dogs.

Benign thyroid tumors were noted only in the two 2-year rat studies. Controls did not have any animals with these tumors. At 22/23 mg/kg in males, a combination of the two studies indicated 3/70 tumors; whereas, in females at 30 mg/kg, the incidence was 1/70 (1/70 also at 6 mg/kg). At the highest dose tested (110-116 mg/kg in males and 150-156 mg/kg in females), the incidences were 5/70 for males and 3/70 for females.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

The CARC concluded that BAS 510 F showed evidence of carcinogenicity based on the following:

- **Evidence of carcinogenicity (thyroid tumors) was seen in both sexes of one species (rat).** When the data were combined from the rat chronic toxicity study and the carcinogenicity study, male rats had a significant increasing trend ($p<0.01$), and significant differences in the pair-wise comparison of the 2500 ppm dose group with the controls for thyroid follicular cell adenomas ($p<0.05$). There was no treatment-related increase in thyroid follicular cell carcinomas. However, when the one follicular cell carcinoma from the control was included, there was only a significant increasing trend for combined adenomas and carcinomas ($p<0.05$) in males. The incidence of thyroid follicular cell adenomas was 0/70, 0/70, 3/70, 5/70 for the 0, 100, 500, and 2500 ppm dose groups, respectively. The incidence at the high dose (2500 ppm; 7% versus 0% in the controls) exceeded the historical control mean (1%) and range (0-6%). The CARC considered the increase in thyroid follicular cell adenomas to be treatment-related in males. This tumor response was supported by thyroid hypertrophy and hyperplasia of follicular cells at 2500 ppm, increased thyroid weights, and mechanistic data.

- When the data were combined from the rat chronic toxicity study and the carcinogenicity study, female rats had a significant increasing trend ($p<0.05$) for thyroid follicular cell adenomas. The incidence of thyroid follicular cell adenomas was 0/70, 1/70, 1/69, 3/70 for the 0, 100, 500, and 2500 ppm dose groups, respectively. The CARC considered the increasing trend for adenomas to be treatment-related in females. This tumor response was consistent with the male response in terms of tumor types and hyperplasia. No carcinomas were observed.

- The highest dose tested of 2500 ppm was considered to be adequate, but not excessive, in both sexes to assess the carcinogenicity of BAS 510 F in Wistar rats. This was based on changes in clinical chemistry/enzymes and organ weights, and liver and thyroid lesions.

- There was no treatment-related increase in any tumors in male and female mice.

- The highest dose tested of 8000 ppm (1345 mg/kg/day in males; 1804 mg/kg/day in females) is above the limit dose in both sexes and, therefore, was considered to be adequate to assess the carcinogenicity of BAS 510 F in C57BL/6 J Rj mice.

2. Mutagenicity

BAS 510 F was not mutagenic in a battery of acceptable *in vitro* and *in vivo* studies.

3. Structure Activity Relationship

No appropriate structural analogues were located for comparison purposes.

4. Mode of Action

The mode of action data were not adequate to characterize the dose-response in relationship to tumor formation. The mechanistic data were useful, however, in the overall weight-of-the-evidence in corroborating the tumor and non-tumor data. Mechanistic studies indicated an interrelationship between the liver and thyroid findings which suggested that the effects on the thyroid may have been the result of a transitory effect on the liver; a reversibility study only in males showed that the dose-effect thyroid parameters returned toward control values during the reversibility phase.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July 1999), the CARC classified BAS 510 F into the category **"Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential"** by the oral route based on the following weight-of-the-evidence considerations:

(i) Evidence of carcinogenicity (thyroid tumors) was seen in both sexes of one species (rat).

(ii) Only benign tumors (thyroid follicular cell adenomas) were seen in male (significant trend and pair-wise at the high dose) and in female rats (trend only).

(iii) The corroborative changes in organ weights, clinical chemistry and non-neoplastic lesions support the thyroid as a target.

(iv) There is no concern for mutagenicity of BAS 510 F.

(v) There is a lack of data on structure activity relationships.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The quantification of human cancer risk is not recommended.

VIII. BIBLIOGRAPHY

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