Reviewer: Brenda MacDonald, D.V.M. , Date: April, 2001

STUDY TYPE: Subchronic One-Year Feeding Toxicity Study in Dogs; OPPTS 870.4100; OECD 452.

TEST MATERIAL (PURITY): BAS 500 F, purity 98.7%

SYNONYMS: Reg. No. 304 428, Pyraclostrobin

CITATION: Schilling, K., et al. (1999). BAS 500 F - Chronic Oral Toxicity Study in Beagle Dogs Administration in the Diet for 12 Months. Department of Toxicology of BASF Aktiengesellschaft, Rhein, FRG. Laboratory Project No. 33D0494/96144, BASF Doc. No. 1999/11677, November 17, 1999. MRID #45118328. Unpublished.

SPONSOR: BASF Canada Inc., Agricultural Products, Toronto, Ontario

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID #45118328), BAS 500 F, purity 98.7%, was administered to 5 beagles/sex/dose in diet at dose levels of 0, 100, 200 and 400 ppm (equal to 0, 2.7, 5.4 and 10.8 mg/kg bw/day for males, and 0, 2.7, 5.4 and 11.2 mg/kg bw/day for females) for a 1-year period. Vomiting was observed in the high dose animals during the first week of the study, which was considered to be due to a transient aversion to the test material in the diet. In addition, diarrhea was observed for all high dose animals, which persisted throughout the study period. For males, a transient loss in body weight was seen during the first week of the study in the 400 ppm group, most likely due to vomiting during study week 1. Thereafter, there was no treatment-related effect on body weight gain (final body weight and overall body weight gain were slightly higher than in the concurrent control group). For females, final body weight and overall body weight gain were lower in the 400 ppm group, due to a net loss in body weight during the first week of the study (likely due to vomiting) and lower body weight gain during the first half of the study. In addition, decreased food intake and decreased food efficiency were seen in the 400 ppm group, females only. Clinical chemistry findings were slightly lower cholesterol, total protein, albumin and globulin in the 400 ppm group, both sexes, which fell at the lower end or just below the normal expected range of values. These findings are considered to reflect a trend towards lower values indicative of a marginal treatment-related effect. Kidney weight was increased in the 400 ppm group, males only. However, in the absence of any corresponding clinical chemistry or histopathological changes, this finding was not considered to be toxicologically significant. There was no treatment-related effect noted at gross necropsy or histopathological examination.

The LOAEL is 400 ppm (equal to 10.8 mg/kg bw/day for males and 11.2 mg/kg bw/day for females), based on an increased incidence of diarrhea and clinical chemistry changes (both sexes) and decreased body weight gain, decreased food intake and decreased food efficiency (females only). The NOAEL is 200 ppm (equal to 5.4 mg/kg bw/day for males and 5.4 mg/kg bw/day for females).

This subchronic toxicity study in the dog is acceptable and satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3150; OECD 409) in dogs

(1)

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** BAS 500 F

Description:	Technical, red-brown clear material
Lot/Batch #:	LJ. -No. 27882/199/b (Tox. III, part 2)
Purity:	98.7 % a.i.
Compound Stability:	Proven by reanalysis after the in-life phase of the study (report of Dec. 8, 1998)
CAS #:	175013-18-0

2. **Vehicle:** Test material dissolved in acetone, then mixed with control diet

3. **Test Animals:**

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	5 to 8 months of age Body weight: Males, 9.2 kg to 12.9 kg; Females, 7.7 kg to 12.9 kg	
Source:	BASF's own Beagle breed	
Housing:	Housed individually in kennels, floor area ~ 5.4 m^2	
Diet:	Dog maintenance Kliba laboratory diet 335 Klingentalmuhle AG, 700 g daily (i.e., 350 g powdered food pellets made into a paste with 350 mL drinking water immediately prior to administration)	
Water:	Blended water (completely demineralized water adjusted with drinking water to ~ 2° German hardness), *ad libitum*	
Environmental conditions:	**Temperature:**	Not stated
	Humidity:	Not stated
	Air changes:	Not stated
	Photoperiod:	Natural day/night rhythm, with additional artificial light as required during working hours
Acclimation period:	7 days	

B. STUDY DESIGN:

1. **In Life Dates:** November 5, 1997 to November 11, 1998.

2. **Animal Assignment**: Animals were assigned to the test groups noted in Table 1 using a random number generator, such that the mean body weights in the individual groups were approximately equal.

TABLE 1: Study Design

Test Group	Conc. in Diet (ppm)	# Male	# Female
Control	0	5	5
Low	100	5	5

Mid	200	5	5
High	400	5	5

3. **Diet Preparation and Analysis:** Diet was freshly prepared every two weeks and stored at room temperature. The test material was frozen and mechanically crushed, then an acetonic solution was made. The solutions were sprayed on ~ 3 kg of diet in a rotation vaporizer. The acetone was removed by heating to ~ 40°C for 30 minutes. The premixes were then adjusted to the desired concentrations with the appropriate amount of food and mixed for 10 minutes in a GEBR.LODIGE laboratory mixer. Test diets were made into a paste immediately prior to administration, i.e., 350 g of powdered food pellets mixed with 350 mL of drinking water. Stability of the test material in diet prepared prior to study initiation was determined after storage at room temperature for 0 and 43 days after preparation at the dose level of 20 ppm. The stability of the test material made into a paste was determined after storage at room temperature (RT) for 0, 1, 3 and 24 hours after preparation at the dose level of 108 ppm. Homogeneity of mixing was determined at dose levels of 100 and 400 ppm for test diets prepared at the beginning of the study and during month 6. The actual test material concentration in the diets was determined for all dose levels, from samples of test diets prepared for the first week of the study, then ~ every 3 months thereafter.

Results:

Stability Analysis: The actual concentrations of BAS 500 F in the test diet, and in the diet paste, at 20 and 108 ppm, respectively, expressed as percentage of the initial concentration, were as follows:

	Dose (ppm)	
Storage Interval	20	108
Day 0	100.0	100.0
Hour 1	----	101.4
Hour 3	----	96.1
Hour 24	----	100.8
Day 43	104.3	----

Based on these results, it was concluded that the stability of the test material in the formulated diets was acceptable according to the use pattern of this study.

Homogeneity Analysis: i) Initial diets: Individual samples of the 100 and 400 ppm test diets ranged from 97.5% to 102.4% and 97.3% to 99.8% of the nominal concentrations, respectively.
ii) Month 6 diets: Individual samples of the 100 and 400 ppm test diets ranged from 91.8% to 97.8% and 91.5 to 94.3% of the nominal concentrations, respectively.

Concentration Analysis: The range of values for the actual concentrations of BAS 500 F in the test diets, and the overall mean values, expressed as percentage of the nominal concentrations, were as follows:

	Dose (ppm)			
	0	100	200	400
Actual concentration, ppm Range of values Mean value	None detected	91.8 - 102.4 96.5	190.0-196.2 193.2	366-399 380
% of target concentration Range of values Mean value	None detected	91.8-102.4 96.5	95.0-98.1 96.6	91.5-99.8 95

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

4. **Statistics**: Statistical analyses performed were as follows:
i) Body weight: Parametric one-way analysis using the F-test (ANOVA). If the resulting p-value was ≤0.05, a comparison of each group with the control group was performed using Dunnett's two-sided test.
ii) Hematology and Clinical Chemistry: Non-parametric one-way analysis using two-sided Kruskal-Wallis test. If the resulting p-value was ≤ 0.05, a pairwise comparison of each dose group with the control group was performed using the two-sided Mann-Whitney U-test.
iii) Urinalysis: Pairwise comparison of each dose group with the control group using Fisher's exact test.
iv) Organ weights: Non-parametric one-way analysis using the two-sided Kruskal-Wallis test. If the resulting p-value was ≤0.05, a pairwise comparison of each dose group with the control group was performed using the Wilcoxon test.

C. METHODS:

1. **Observations**: Animals were examined twice daily for mortality and moribundity and at least once daily for clinical signs of toxicosis.

2. **Body Weight**: Animals were weighed on a weekly basis.

3. **Food Consumption and Compound Intake**: Food consumption was determined on a daily basis. From the body weight and food intake data, the daily substance intake per animal (mg/kg bw/day) and the weekly feed efficiency per animal (weekly body weight change, g/weekly food consumption, g x 100) were calculated.

4. **Ophthalmoscopic Examination**: An ophthalmological examination was conducted on all animals prior to study initiation and on study day 364.

5. **Haematology & Clinical Chemistry**: Blood samples were taken 9 days (males) or 8 days (females) prior to study initiation, and on study days 89, 180 and 362 for males, and study days 90, 181 and 363 for females via the vena cephalica antebrachii, for haematology and clinical chemistry analysis. Animals were fasted overnight prior to collection. The CHECKED (✓) parameters were examined.

a. Haematology

✓	Hematocrit (HCT)	✓	Leukocyte differential count
✓	Hemoglobin (HGB)	✓	Mean corpuscular HGB (MCH)
✓	Leukocyte count (WBC)	✓	Mean corpusc. HGB conc.(MCHC)
✓	Erythrocyte count (RBC)	✓	Mean corpusc. volume (MCV)
✓	Platelet count		Reticulocyte count
	Blood clotting measurements		RBC morphology
✓	(Activated Partial Thromboplastin time)		
	(Clotting time)		
✓	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
✓	Calcium	✓	Albumin
✓	Chloride	✓	Blood creatinine
✓	Magnesium	✓	Blood urea nitrogen
✓	Phosphorus	✓	Total Cholesterol
✓	Potassium	✓	Globulin
✓	Sodium	✓	Glucose
	ENZYMES	✓	Total bilirubin
✓	Alkaline phosphatase (ALK)	✓	Total serum protein (TP)
✓	Cholinesterase (ChE)	✓	Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		A/G ratio
✓	Serum alanine amino-transferase (also SGPT)		
✓	Serum aspartate amino-transferase (also SGOT)		
	Sorbitol dehydrogenase		
✓	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

6. Urinalysis: Thirteen days (males) or twelve days (females) prior to study initiation, and on study days 92, 183 and 358 for males and study days 93, 184 and 359 for females, animals were placed in individual metabolism cages. Urine was then collected overnight, during which time food was withheld. Each animal was provided with ~ 500 mL of water. The CHECKED (✓) parameters were examined.

✓	Appearance	✓	Glucose
✓	Volume	✓	Ketones
✓	Specific gravity / osmolality	✓	Bilirubin
✓	pH	✓	Blood / blood cells
✓	Sediment (microscopic)	✓	Nitrate
✓	Protein	✓	Urobilinogen

7. Sacrifice and Pathology: At study termination, animals were anesthetized (method not stated) and sacrificed by exsanguination, then necropsied. The CHECKED (✓) tissues were collected and preserved in 4% formaldehyde solution for histological examination. In addition, the (✓✓) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	✓	Aorta	✓✓	Brain
✓	Salivary glands	✓	Heart	✓	Peripheral nerve
✓	Esophagus	✓	Bone marrow	✓	Spinal cord (3 levels)
✓	Stomach	✓	Lymph nodes	✓	Pituitary
✓	Duodenum	✓	Spleen	✓	Eyes (optic nerve)
✓	Jejunum	✓	Thymus		GLANDULAR
✓	Ileum			✓✓	Adrenal gland
✓	Cecum		UROGENITAL		Lacrimal gland
✓	Colon	✓✓	Kidneys	✓	Mammary gland
✓	Rectum	✓	Urinary bladder	✓✓	Parathyroid
✓✓	Liver	✓✓	Testes	✓✓	Thyroid
✓	Gall bladder	✓✓	Epididymides		OTHER
✓	Pancreas	✓	Prostate	✓	Bone
	RESPIRATORY	✓✓	Ovaries	✓	Skeletal muscle
✓	Trachea	✓	Uterus	✓	Skin
✓	Lung	✓	Vagina	✓	All gross lesions and masses
	Nose	✓	Oviducts		
	Pharynx	✓	Ureter		
	Larynx				

The (✓) tissues were examined from all animals in the control, 100, 200 and 400 ppm groups.

II. RESULTS

A. Observations:

1. **Mortality:** All animals survived the duration of the study period.

2. **Clinical Observations:** An increased incidence of diarrhea in the 400 ppm group, observed in all males and females throughout the study period, was considered to be treatment-related. The duration of the diarrhea for individual animals in the 400 ppm group, were as follows: for males, 41 weeks , 32 weeks, 42 weeks, 20 weeks and 4 weeks, and for females, 4 weeks, 19 weeks, 42 weeks, 11 weeks and 11 weeks. Diarrhea was also noted in the 100 and 200 ppm group, but was not considered to be treatment-related due to the isolated occurrence, i.e., in the 100 ppm group, 2 of 5 males were affected (during week 0 and week 27, repectively), and 1 of 5 females was affected (during week 29), and in the 200 ppm group, 1 male was affected (during week 16 and 31) and 1 female was affected (week 1). The only other finding considered to be treatment-related was vomiting noted for 3 males and 4 females during the first study week in the 400 ppm group. This was considered to be due to a transient aversion to the test material in the diet. A single incident of vomiting in the 100 ppm group (week 25) was not considered to be treatment-related.

B. Body Weight and Weight Gain: Refer to Table 2.
i) Males: A net loss in body weight was seen during the first week of the study in the 400 ppm group, likely attributable to the vomiting observed during study week 1. Thereafter, there was no treatment-related effect on body weight gain. Final body weight and overall body weight gain were slightly higher

in the 400 ppm group than in the concurrent control group.
There was no treatment-related effect on body weight gain in the 100 and 200 ppm groups.
ii) Females: Final body weight and overall body weight gain were lower in the 400 ppm group, due to a net loss in body weight during the first week of the study and lower body weight gain during the first half of the study. Body weight loss during the first week of the study was likely in part due to vomiting and decreased food intake during study week 1. There was no treatment-related effect on body weight gain in the 100 and 200 ppm groups.

TABLE 2. Mean Body Weights and Body Weight Gains (kg)[a]

Time Interval	Dose (ppm)			
	0	100	200	400
Males				
Week 0	11.0±1.5	11.2±1.2	10.9±1.2	11.0±1.3
Week 1	11.4±1.3	11.4±1.2	11.2±1.1	10.9±1.4
Week 6	12.4±1.0	12.4±1.2	12.4±1.0	11.9±0.9
Week 13	13.1±0.9	12.9±1.1	13.1±1.0	12.5±1.0
Week 26	13.3±0.9	12.9±0.9	13.2±1.4	12.9±0.9
Week 52 (% of control)	12.9±1.1 ---	12.7±0.9 (98.4)	13.3±1.4 (103.1)	13.2±0.8 (102.3)
bw gain, wk 0-1	0.4	0.2	0.3	-0.1
bw gain, wk 1-6	1.0	1.0	1.2	1.0
bw gain, wk 6-13	0.7	0.5	0.7	0.6
bw gain, wk 13-26	0.2	0.0	0.1	0.4
bw gain, wk 26-52	-0.4	-0.2	0.1	0.3
Total gain (% of control)	1.9 ---	1.5 (78.9)	2.4 (126.3)	2.2 (115.8)
Females				
Week 0	10.0±1.7	10.2±1.0	10.5±1.5	10.1±1.1
Week 1	10.2±1.7	10.5±0.9	10.8±1.4	9.9±1.1
Week 6	11.2±1.6	11.7±0.9	11.7±1.4	10.4±1.6
Week 13	11.7±1.7	12.3±0.9	12.3±1.7	10.5±1.2
Week 26	12.2±1.7	12.8±0.9	12.2±1.6	10.4±1.8
Week 52 (% of control)	12.7±2.1 ---	13.4±1.3 (105.5)	12.9±1.3 (101.6)	11.2±1.6 (88.2)
bw gain, wk 0-1	0.2	0.3	0.3	-0.2

bw gain, wk 1-6	1.0	1.2	0.9	0.5
bw gain, week 6-13	0.5	0.6	0.6	0.1
bw gain, week 13-26	0.5	0.5	-0.1	-0.1
bw gain, week 26-52	0.5	0.6	0.7	0.8
Total gain (% of control)	2.7 —	3.2 (118.5)	2.4 (88.9)	1.1 (40.7)

[a] Data obtained from pages 151 to 163 in the study report.
* Significantly different ($p < 0.05$) from the control.

C. Food Consumption and Compound Intake:

1. Food Consumption: Total mean food intake was lower in the 400 ppm group, females only, due to decreased food intake throughout the study period.
Total mean food intake values (daily mean value; percent of control group value in brackets) for the 0, 100, 200 and 400 ppm groups, respectively, were as follows:
i) For males: 250292 g (699.1 g), 250600 g (700 g; 100.1%), 250600 g (700 g; 100%) and 245357 g (685.4 g; 98.0%); and,
ii) For females: 244075 g (679.9 g), 242593g (675.8 g; 99.4%), 239910 g (668.3; 98.3%) and 216383 g (602.7 g; 88.7%).

Mean food intake was comparable amongst the 0, 100 and 200 ppm groups.

2. Compound Consumption: Based on food consumption, the nominal dietary concentrations and body weights, the doses expressed as mean daily mg test substance/kg body weight during the study period for the 0, 100, 200 and 400 ppm groups, respectively, were as follows:
i) For males: 0, 2.7, 5.4 and 10.8 mg/kg bw/day; and,
ii) For females: 0, 2.7, 5.4 and 11.2 mg/kg bw/day.

3. Food Efficiency: i) Males: There was no treatment-related effect on food efficiency at any dose level tested. Overall food efficiency values for the 0, 100, 200 and 400 ppm groups were 1.36, 1.13, 1.71 and 1.80, respectively.
ii) Females: Food efficiency was markedly lower in the 400 ppm group throughout most of the study period, resulting in lower overall feed efficiency, i.e., overall food efficiency values for the 0, 100, 200 and 450 ppm groups were 1.77, 2.32, 1.63 and 0.93, respectively.
This effect was most pronounced during the first week of the study, i.e., food efficiency values for study week 1 were 8.2, 13.9, 9.8 and -11.6 for the 0, 100, 200 and 400 ppm groups, respectively.

There was no treatment-related effect on feed efficiency in the 100 and 200 ppm groups.

D. Ophthalmoscopic Examination: There were no treatment-related findings.

E. Blood Analyses

1. Haematology: Refer to Tables 3 and 4.
i) Males: Findings considered to be treatment-related by the study author were increased platelet count at

all time intervals in the 400 ppm group, and increased WBC count, resulting from an increase in the absolute number of neutrophils and lymphocytes, at 180 and 362 days in the 400 ppm group. However, this group had higher platelet and WBC counts at all time intervals measured, including pre-treatment measurement, there was no dose-response relationship, the increase in neutrophil and lymphocyte counts were not statistically significant and all values fell within the normal expected range. Hence, the PMRA reviewer does not consider these findings to be treatment-related but rather reflect normal variation.
ii) Females: The study author considered an increase in platelet count in the 400 ppm group at all time intervals to be treatment-related. However, this finding was not statistically significant, and all values fell within the normal expected range of values, and so the PMRA reviewer does not consider this to be treatment-related but rather reflects normal variation.

There were no other findings considered to be related to treatment with BAS 500 F.

TABLE 3 - Selected Hematology Findings[a], males

	Dose (ppm)			
Parameter	**0**	**100**	**200**	**400**
Platelets, GIGA/L: day -9	303±62	319±66	267±36	382±49
day 89	268±34	284±37	253±30	366±44**
day 180	283±30	290±39	266±50	348±20**
day 362	304±28	274±30	267±44	392±58**
WBC, GIGA/L: day -9	9.49±1.53	11.30±1.58	8.33±1.08	12.33±3.69
day 89	9.93±1.51	11.47±1.82	11.23±2.21	12.15±1.65
day 180	8.98±1.01	10.30±1.20	9.61±1.96	12.81±2.17**
day 362	8.26±0.43	9.32±1.13*	8.70±1.43	12.71±1.07**
Neutrophils, GIGA/L: day -9	4.67±0.71	5.47±1.03	4.33±0.76	6.16±1.97
day 89	5.48±0.87	6.34±1.51	6.80±1.86	6.40±0.99
day 180	5.08±0.62	5.75±1.32	5.85±1.33	7.19±1.07
day 362	5.01±0.66	5.54±1.18	5.42±0.91	7.01±0.62
Lymphocytes, GIGA/L: day -9	3.90±1.10	4.82±0.76	3.32±0.50	4.88±1.21
day 89	3.58±0.65	4.25±0.47	3.42±0.53	4.86±1.01
day 180	3.08±0.43	3.74±0.47	3.00±0.53	4.58±1.54
day 362	2.39±0.33	3.10±0.24	2.51±0.49	4.61±0.98

[a] Data obtained from pages 199 to 225 in the study report.
* statistically significantly different from control, $p < 0.05$

TABLE 4 - Selected Hematology Findings[a], females

Parameter	Dose (ppm)			
	0	100	200	400
Platelets, GIGA/L: day -8	312±62	325±65	331±26	332±37
day 90	295±54	272±56	331±65	351±68
day 181	297±61	306±41	335±72	386±103
day 363	319±60	302±68	349±91	401±89

[a] Data obtained from pages 199 to 225 in the study report.
* statistically significantly different from control, $p < 0.05$

2. <u>Clinical Chemistry</u>: Refer to Tables 5, 6 and 7. Findings considered to be treatment-related were decreased cholesterol, total protein, albumin and globulin, noted in the 400 ppm group, both sexes, at all time intervals. The cholesterol values fell at the lower end of the normal expected range of values, and total protein, albumin and globulin values fell at the lower end or just below the normal expected range of values. Hence, the PMRA reviewer concludes that the above-noted changes reflect a trend towards decreased values and a marginal treatment-related effect.
In addition, glucose was slightly decreased in the 400 ppm group at 89/90 days, both sexes. This was not considered to be treatment-related since the values fell within the normal expected range of values and other time intervals were not similarly affected.

TABLE 5 - Selected Clinical Chemistry Findings[a], after 89/90 days of treatment

Parameter	Dose (ppm)			
	0	100	200	400
Cholesterol, mmol/L - males	4.78±0.33	5.23±0.74	4.47±0.85	3.34±0.30**
- females	4.54±0.98	4.63±0.30	4.28±0.74	3.38±0.53
Total protein, g/L - males	58.30±1.39	59.03±2.78	56.85±3.06	52.50±1.10**
- females	58.04±3.50	58.56±2.34	57.09±2.90	51.38±3.48**
Albumin, g/L - males	34.92±0.29	34.95±1.03	34.41±0.86	31.69±1.31**
- females	35.57±2.69	36.44±1.14	34.91±2.42	31.95±2.37
Globulin, g/L - males	23.39±1.55	24.09±1.99	22.43±2.71	20.91±1.97
- females	22.48±1.10	22.12±1.99	22.18±0.83	19.43±1.72
Glucose, mmol/L - males	6.06±0.16	5.95±0.26	6.01±0.38	5.75±0.33
- females	6.21±0.27	6.20±0.29	5.97±0.63	5.69±0.32**

[a] Data obtained from pages 233 to 248 in the study report.
* statistically significantly different from control, $p < 0.05$
** statistically significantly different from control, $p < 0.01$

TABLE 6 - Selected Clinical Chemistry Findings[a], after 180/181 days of treatment

Parameter	Dose (ppm)			
	0	100	200	400
Cholesterol, mmol/L - males	4.31±0.50	4.77±0.18	4.42±0.78	3.21±0.36**
- females	4.79±0.76	5.50±1.75	4.07±0.86	3.09±0.53**
Total protein, g/L - males	57.31±0.91	58.36±1.35	58.31±2.13	53.07±1.39**
- females	57.43±1.98	59.54±2.09	56.37±3.20	51.49±4.53**
Albumin, g/L - males	30.18±0.60	30.25±0.79	30.58±0.50	27.88±0.51**
- females	30.50±2.34	31.03±1.74	30.21±1.83	27.65±4.19
Globulin, g/L - males	27.13±1.28	28.11±1.00	27.74±1.74	25.20±1.40
- females	26.93±0.70	28.50±1.66	26.16±2.00	23.84±0.53**

[a] Data obtained from pages 233 to 248 in the study report.
* statistically significantly different from control, $p < 0.05$
** statistically significantly different from control, $p < 0.01$

TABLE 7 - Selected Clinical Chemistry Findings[a], after 362/363 days of treatment

Parameter	Dose (ppm)			
	0	100	200	400
Cholesterol, mmol/L - males	4.57±0.70	4.58±0.32	4.38±0.63	3.16±0.36**
- females	4.60±0.93	5.41±2.08	4.43±0.90	3.23±0.47**
Total protein, g/L - males	61.82±1.85	61.64±2.77	62.00±3.25	53.52±4.06**
- females	62.02±2.70	63.34±2.29	59.38±3.27	55.41±2.52**
Albumin, g/L - males	31.00±1.11	30.16±0.83	31.12±0.83	26.96±2.29**
- females	30.93±1.90	32.43±0.98	30.59±2.25	29.53±2.53
Globulin, g/L - males	30.82±2.47	31.48±2.52	30.88±2.60	26.55±1.93
- females	31.09±2.67	30.91±2.84	28.79±1.51	25.88±0.99**

[a] Data obtained from pages 233 to 248 in the study report.
* statistically significantly different from control, $p < 0.05$
** statistically significantly different from control, $p < 0.01$

F. Urinalysis: There were no treatment-related findings.

G. Sacrifice and Pathology:

1. Organ Weight: Refer to Tables 8 and 9. For males, the absolute and relative kidney weights were increased at all dose levels. However, the values fell within the historical control range of values

provided by the registrant. In addition, there were no corresponding histopathological or clinical chemistry changes, and so the increased kidney weights were not considered to be toxicologically significant. The only other finding was decreased absolute liver weight in the 400 ppm group, both sexes. However, these findings were not statistically significant and there were no corresponding histopathological or clinical chemistry changes and so were not considered to be treatment-related.

TABLE 8 - Selected Organ Weights [a] - males, absolute (g) and relative (% bw)

	Dose (ppm)			
	0	100	200	400
Kidneys - absolute	54.09±5.40	62.88±6.82**	65.03±4.78*	65.41±7.83*
- relative	0.418±0.043	0.500±0.078	0.489±0.037	0.499±0.046
Liver - absolute	383.01±42.22	388.22±41.96	369.62±50.18	349.29±39.10
- relative	2.99±0.51	3.06±0.21	2.76±0.16	2.66±0.17

[a] Data obtained from pages 265 to 268 in the study report.

Historical Control Data for kidney weights of male beagle dogs:
Data was obtained from 21 one-year dog feeding studies conducted between 1986 and 1997, 5 or 6 dogs per study (total of 122 dogs) The range for mean absolute kidney weight was 52.26±4.63 g to 75.66±8.63 g, mean 61.33±5.66 g, and for mean relative kidney weight was 0.422±0.041% to 0.575±0.067, mean 0.493±0.035.

TABLE 9 - Selected Organ Weights [a] - females, absolute (g) and relative (% bw)

	Dose (ppm)			
	0	100	200	400
Liver - absolute	343.56±49.28	386.71±28.92	366.76±44.02	282.25±39.01
- relative	2.73±0.27	2.87±0.09	2.82±0.25	2.55±0.29

[a] Data obtained from pages 265 to 268 in the study report.

2. **Gross Pathology:** There were no treatment-related findings.

3. **Microscopic Pathology:** There were no treatment-related findings.

III. DISCUSSION

A. **Investigators' Conclusions:** "In conclusion, the following substance-related adverse effects were obtained:
400 ppm: body weight loss in the females up to study day 21, thereafter retarded body weight gain up to the end of the administration period; reduced food consumption in the females; reduced food efficiency in the females; initial vomitus in both sexes; diarrhea in both sexes; decreases in total protein, albumin, glogulins and cholesterol in both sexes; increases in platelets in both sexes; and increases in white blood cells, polymorphonuclear neutrophils and lymphocytes in the males.
200 ppm: no substance-related adverse effects.
100 ppm: no substance-related adverse effects.

Thus, substance-related adverse effects were seen at the high dose of 400 ppm, only. Toxicity was

mainly characterized by body weight loss and retarded body weight gain, reduced food consumption and reduced food efficiency in females. Moreover, diarrhea and related changes in clinical chemistry like hypoproteinemia occurred in both sexes. Further findings in clinical pathology consisted of an increase platelet count as well as slight increase in white blood cells indicating a mild inflammatory reaction in the males although no morphological correlate was noted. Regarding pathology the oral administration of the test substance did not result in treatment-related weight changes, gross lesions or microscopic findings in any of the dogs investigated. The administration of 100 ppm and 200 ppm was tolerated by the male and female dogs without any adverse changes related to the test substance administered.

The no observed adverse effect level (NOAEL) for male and female Beagle dogs under the conditions of the study was 200 ppm (males and females: 5.4 mg/kg bw/day)."

B. Reviewer's Comments: Male and female beagle dogs were fed test diets containing BAS 500 F, purity 98.7%, at dose levels of 0, 100, 200 and 400 ppm (equal to 0, 2.7, 5.4 and 10.8 mg/kg bw/day for males, and 0, 2.7, 5.4 and 11.2 mg/kg bw/day for females) for a period of 1 year, 5 dogs per sex per group. Vomiting was observed in the high dose animals during the first week of the study, which was considered to be due to a transient aversion to the test material in the diet. In addition, diarrhea was observed for all high dose animals, which persisted throughout the study period. A slight increase in the incidence of diarrhea noted in the 100 and 200 ppm groups was not considered to be toxicologically significant because of its isolated occurrence. For males, a transient loss in body weight was seen during the first week of the study in the 400 ppm group, most likely due to vomiting during study week 1. Thereafter, there was no treatment-related effect on body weight gain (final body weight and overall body weight gain were slightly higher than in the concurrent control group). For females, final body weight and overall body weight gain were lower in the 400 ppm group, due to a net loss in body weight during the first week of the study (likely due to vomiting) and lower body weight gain during the first half of the study. In addition, decreased food intake and decreased food efficiency were seen in the 400 ppm group, females only. Slightly increased platelet count was noted in the 400 ppm group, both sexes. However, values fell within the normal expected range and are considered to reflect normal biological variation. In addition, increased WBC count, due to an increase in the absolute number of neutrophils and lymphocytes, was noted at 180 and 362 days for males in the 400 ppm group. However, this group had higher WBC counts at all time intervals measured, including pre-treatment measurement, there was no dose-response relationship, the increase in neutrophil and lymphocyte count were not statistically significant and all values fell within the normal expected range. Hence, these findings are not considered to be treatment-related but rather reflect normal variation. Clinical chemistry findings were slightly lower cholesterol, total protein, albumin and globulin, in the 400 ppm group, both sexes. Values for these parameters fell at the lower end or just below the normal expected range of values and are considered to reflect a trend towards lower values indicative of a marginal treatment-related effect. Kidney weight was increased in the 400 ppm group, males only. However, in the absence of any corresponding clinical chemistry or histopathological changes, this finding was not considered to be toxicologically significant. There was no treatment-related effect at gross necropsy or histopathological examination.

Based on the results of this study, the LOAEL was determined to be 400 ppm (equal to 10.8 mg/kg bw/day for males and 11.2 mg/kg bw/day for females) based on an increased incidence of diarrhea and clinical chemistry changes (both sexes), and decreased body weight gain, decreased food intake and decreased food efficiency (females only). The NOAEL was 200 ppm (equal to 5.4 mg/kg bw/day for males and females).

C. Study Deficiencies: No scientific deficiencies were noted which would compromise the interpretation of the study.