1/3/97

DATA EVALUATION REPORT

AZOXYSTROBIN

STUDY TYPE: SUBCHRONIC ORAL TOXICITY [CAPSULE] - DOG (82-1(b))

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 95-190

Primary Reviewer: S. Milanez, Ph.D.	Signature: Date:	S. Milanez 3-12-96
Secondary Reviewers: S. Talmage, Ph.D., D.A.B.T.	Signature: Date:	Sylvia Talmage 3/14/96
Robert H. Ross, Group Leader	Signature: Date:	Robert H. Ross 3-12-96
Quality Assurance: Susan Chang, M.S.	Signature: Date:	S V S Chang 3-12-96

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EPA Reviewer:
Myron S. Ottley, Ph.D. MSOttley, Date 12/31/96
Review Section IV, Toxicology Branch I(7509C)
EPA Secondary Reviewer:
Marion Copley, D.V.M., D.A.B.T. MCopley, Date 1/3/97
Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [capsule] - Dog
OPPTS 870.3150 [§82-1(b)]

DP BARCODE: D218319 SUBMISSION CODE: S489692
P.C. CODE: 128810 TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): ICIA5504 (AZOXYSTROBIN) (96.2% w/w)
SYNONYMS: methyl (E)-2-(2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl)-3-methoxyacrylate

CITATION: Allen, S. (1995) ICIA5504: 90 Day oral dosing study in dogs. Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Report number CTL/P/3890, May 11, 1993. MRID 43678136. Unpublished.

SPONSOR: Zeneca Inc. Agricultural Products, Wilmington, DE 19897.

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID 43678136) ICIA5504 (96.2% w/w) was administered to 4 beagle dogs/sex/dose by capsule at doses of 0, 10, 50, or 250 mg/kg/day for 92 or 93 days (equal numbers of dogs in each dose group were treated for each number of days).

No animals died during the study. Treatment-related clinical observations in both sexes included increases in salivation at dosing, fluid feces, vomiting, and regurgitation primarily at 250 mg/kg/day (statistical analysis was not performed). These signs may have contributed to the lowered animal weights. The weekly body weights of both sexes differed statistically from controls for most weeks at 250 mg/kg/day and in females at 50 mg/kg/day, though values were within 9% of controls ($p \leq 0.05$ or 0.01). Total body weight gains were 34% and 38% lower than controls in high dose males and females, respectively. Hematological alterations at 250 mg/kg day in one or both sexes were small (< 9% change) compared to concurrent controls and/or pre-treatment values and not toxicologically relevant. Clinical chemistry parameters that were altered significantly from controls ($p \leq 0.05$ or 0.01) at the high dose in both sexes during one or more weeks include plasma cholesterol (13-26% increase), triglycerides (42-89% increase), alkaline phosphatase (24-87% increase), and plasma albumin (7.9-11.6% decrease). Cholesterol was increased in mid- and low-dose males (17-25%). These results were accompanied by increased

absolute liver weight in mid- and high-dose females (6.3%, $p \leq 0.05$; 9.3%, $p \leq 0.01$, respectively), and are consistent with an adverse effect on liver and possibly biliary function. The lack of histopathological correlates and of a clearly dose- and time-related response in some cases, indicated the clinical and liver weight changes were an adaptive liver response. Other clinical chemistry changes ($p \leq 0.05$ or 0.01) did not appear to be treatment-related (plasma sodium, creatinine, and total protein). There were no treatment-related effects on gross or microscopic pathology, food consumption, ophthalmology, or urinalysis. The increased thyroid weight, in the absence of histologic change, in high-dose females (37%, $p \leq 0.01$) was of uncertain toxicological significance. **The LOAEL (lowest observeable adverse effect level) is 250 mg/kg/day for both male and female dogs under the conditions of this study, based on treatment-related clinical observations and clinical chemistry alterations at this dose. The NOAEL is 50 mg/kg/day.**

This subchronic toxicity study is classified acceptable and satisfies the guideline requirement for a subchronic oral study (82-1b) in dogs.

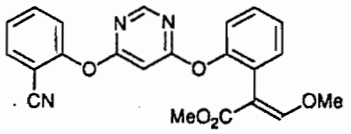
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: ICIA5504

   Description: light brown solid; unformulated technical material
   Lot/Batch #: Y06654/014/P49
   Purity: 96.2% w/w
   Stability of compound: stable at room temperature in dark, vented area
   CAS #: not available
   Structure:



2. Vehicle and/or positive control: none; negative controls received empty capsules

3. Test animals

Species: dog
Strain: beagle
Age and weight at study initiation: 18-21 weeks at receipt (plus a 4-5 week acclimatization period). On treatment day 1, weight ranges were: 10.3-13.1 kg for males; 9.7-11.9 kg for females.
Source: Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, U.K.
Housing: 365 x 115 cm indoor pens with heated sleeping area. Dogs were housed in pairs or threes for at least 7 days, and individually thereafter.
Diet: Male dogs were fed 400 g and females 350 g of an expanded dry diet (Laboratory Diet A from Special Diets Services Ltd, Stepfield, Witham, Essex, U.K.).
Water: Potable water was supplied *ad libitum*.
Environmental conditions:
Temperature: 21-24°C
Humidity: not specified
Air changes: 10/hour
Photoperiod: 12 hours light per 24 hours
Acclimation period: 4-5 weeks

B. STUDY DESIGN

1. In life dates

Start: June, 1992; end: September, 1992

2. Animal assignment

Animals were assigned randomly to the test groups in Table 1, such that there was an even distribution according to litter and body weight among the groups.

TABLE 1: Study design

Test Group	Dose to Animal (mg/kg/day)	Number of Animals	
		Male	Female
Control	0	4	4
Low	10	4	4
Mid	50	4	4
High	250	4	4

Data taken from p. 13, MRID 43678136.

3. Dose selection rationale

Dose levels were selected based on results from a six-week oral dose ranging study performed at the same laboratory as the present study. No further details were given by the study author.

4. Diet preparation and analysis

The test material was administered daily in gelatin capsules which were loaded for individual animals with dosages based on their most recent body weight and a purity of 96.2% w/w. It was not specified when the gelatin capsules were prepared.

Results -
Homogeneity Analysis: Not applicable; test material was administered in gelatin capsules.

Stability Analysis: Not applicable; test material administered in gelatin capsules. The test material itself was noted by the study author as being stable throughout the course of the study, although details were not provided.

Concentration Analysis: Not applicable; test material (reported as being 96.2% pure) was administered in gelatin capsules.

5. Statistics

Statistical analysis was done by analysis of either variance or covariance using the GLM procedure in SAS (SAS Institute Inc. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2, Cary, NC: SAS Institute Inc, 1989). The differences from the controls of the treated groups were represented by the differences in their least-squares means, and were tested statistically using a two-sided Student's t-test based on the error mean square in the analysis. Body weight changes were considered by analysis of covariance from day 1 weights, separately for males and females. Hematology and blood clinical chemistry values were compared to pre-experimental values by analysis of covariance, for males and females together; a covariate adjustment was also made based on separate sex pre-experimental group means. The plasma gamma-glutamyl transferase activity and plasma total bilirubin were not analyzed statistically. Urine clinical chemistry and organ weight differences from controls were examined by analysis of variance; male and female data were analyzed together for the former, and separately for the latter.

C. METHODS

1. Observations

Animals were inspected at least twice daily for signs of toxicity and mortality. A full clinical examination was performed at week 1 and at termination.

2. Body weight

Animals were weighed before feeding on treatment day 1 and weekly throughout the pre-experimental and treatment periods.

3. Food consumption and compound intake

Food consumption for each animal was determined daily by measuring the food residues (which were thrown away) before each morning feeding. Food efficiency was not calculated by the authors, but was calculated by the reviewer as [body weight gain (kg)/food consumption (kg) X 100] for the total treatment time (92.5 days average). Food residues were subtracted from the daily intake used to calculate the food efficiencies. The compound was administered by gelatin capsules based on animal body weight (mg/kg/day), and was independent of food consumption.

4. Ophthalmoscopic examination

Eyes were examined by indirect ophthalmoscopy at week 1 and prior to termination.

5. Blood was collected from the jugular vein of all surviving animals before the morning feeding (i.e. after overnight fast) at weeks -1, 4, 8, and 13 for hematology and clinical analysis. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*		Reticulocyte count
x	Blood clotting measurements*		
x	(Thromboplastin time)		
x	(Kaolin-cephalin time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical chemistry

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

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was collected by catheterization from animals pre-experimentally and in the week prior to termination. The CHECKED (X) parameters were examined.

Xx		X	
x	Appearance	x	Glucose
x	Volume	x	Ketones
x	Specific gravity	x	Bilirubin
x	Ph	x	Blood
x	Sediment (microscopic)		Nitrate
	Protein	x	Urobilinogen

* Not required for subchronic studies

7. Sacrifice and pathology

All animals survived to study termination, at which time they were sacrificed by sodium pentobarbitone anesthesia and exsanguination, and were subjected to gross pathological examination. The CHECKED (X) tissues were collected and examined histologically. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
x	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	x	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)[T]
x	Stomach*	x	Lymph nodes*		
x	Duodenum*	x	Spleen*	x	Pituitary*
x	Jejunum*	x	Thymus*	x	Eyes (optic n.)[T]
x	Ileum*				
x	Cecum*		UROGENITAL		GLANDULAR
x	Colon*	xx	Kidneys*+	xx	Adrenal gland*
x	Rectum*	x	Urinary bladder*		Lacrimal gland[T]
xx	Liver*[+]	xx	Testes*[+]	x	Mammary gland[T]
x	Gall bladder*	xx	Epididymides	x	Parathyroids*[++]
x	Pancreas*	x	Prostate	xx	Thyroids*[++]
			Seminal vesicle		
	RESPIRATORY	x	Ovaries		OTHER
x	Trachea*	x	Uterus*	x	Bone
x	Lung*			x	Skeletal muscle
	Nose			x	Skin
	Pharynx			x	All gross lesions and masses*
	Larynx				

*Required for subchronic studies based on Subdivision F Guidelines
[+]Organ weight required in subchronic and chronic studies.
[++]Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

II. RESULTS

A. OBSERVATIONS

1. Toxicity

In both sexes of high-dose dogs, there were marked increases in salivation at dosing, fluid feces, vomiting, and regurgitation. The study author did not analyze the results statistically, however, the effects appeared to be dose-related particularly for salivation at dosing and fluid feces. There was no clear temporal pattern (Table 2). Because there were no macroscopic or microscopic correlates, however, the clinical signs are not considered toxicologically significant.

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TABLE 2: Clinical observations in dogs given ICIA5504

Dose (mg/kg/day)	Males			Females		
	No. of affected animals	Study weeks affected	Total number of occurrences	No. of affected animals	Study weeks affected	Total number of occurrences
Salivation						
0	0/4	-	0	0/4	-	0
10	0/4	-	0	0/4	-	0
50	1/4	5-8,13	5	1/4[1]	4,5,8[1]	3[1]
250	4/4	3-13	21	3/4[1]	2-4,6-13[1]	19[1]
Salivation at dosing •						
0	0/4	-	0	0/4	-	0
10	1/4	10	1	0/4	-	0
50	2/4	3-8, 10,12,13	39	0/4	-	0
250	4/4	2-14	245	3/4	2-13	185
Fluid feces						
0	2/4	1-3,13	8	2/4	1,3,7	4
10	3/4	1-8,10	25	4/4	2,3,7,13	4
50	3/4	1-6, 8-13	30	3/4	1-6,10,12	20
250	4/4	1-14	158	4/4	1-13	112
Vomiting and Regurgitation (combined)						
0	0/4	-	0	2/4	2,6	2
50	2/4	1,4,7	4	0/4	-	0
250	3/4	1,5,12,13	8	4/4	1-3,9	11

Data taken from pages 30-41, 111-142, and 165-173, MRID 43678136.
[1] The incidence and frequencies include those of the clinical observation signs of salivation (in addition to salivation).

2. Mortality

All dogs survived to the scheduled termination date.

B. BODY WEIGHT AND WEIGHT GAIN

In male dogs, there were statistically significant decreases in the body weights that were in all cases within 6% of controls (Table 3). In males given 250 mg/kg/day, statistical significance at the 1% level was attained in 10 of the 12 weeks between weeks 3-14, and for 50 mg/kg/day males, significance at the 5% level was attained in week 13. For females, there were statistically significant decreases in body weight at almost all weeks throughout the experiment, both at 50 and 250 mg/kg/day, but they were in all cases within 9% of controls. Significance was at the 5% level during all but three weeks (3, 5, and 7) at 250 mg/kg/day. The net weight gains of males were 16%, 16%, and 34% lower than controls, and of females were 29%, 33%, and 38% lower at 10, 50, and 250 mg/kg/day, respectively, at the end of the treatment period. Statistical analysis was not performed on these values. The body weight depressions do not appear be of major toxicologically significance, and may be partly due to the increased frequency of fluid feces, vomiting and regurgitation in the treated animals.

TABLE 3: Mean body weights (kg) in dogs given ICIA5504

Week	Dose - Males (mg/kg/day)				Dose - Females (mg/kg/day)			
	0	10	50	250	0	10	50	250
1	11.7	11.8	11.8	12.0	10.8	10.9	10.8	10.6
4	12.7	12.7	12.6	12.5**	11.5	11.5	11.1*	10.9*
6	13.3	13.2	13.2	13.0**	11.9	11.7	11.5*	11.2*
8	13.8	13.7	13.7	13.4**	12.2	11.9	11.7*	11.5*
10	14.1	14.1	14.0	13.5**	12.5	12.1	11.9*	11.6*
12	14.6	14.3	14.2	13.9**	12.8	12.3	12.0*	11.7*
14	14.9	14.5	14.5	14.1**	12.9	12.4	12.2	11.9*
Net gain (kg)[1]	3.2	2.7	2.7	2.1	2.1	1.5	1.4	1.3

Data taken from pages 43 and 44, MRID 43678136.
*Statistically different from control group mean at the 5% level;
**Statistically different from control group mean at the 1% level
[1] Values were not analyzed statistically.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Dogs of both sexes generally ate all their allocated food every day. Exceptions were 1 female at 250 mg/kg/day (weeks 1 and 3), and 3 males at 250 mg/kg/day (during 1 or more of the first three weeks, one dog additionally at weeks 4-9).

2. Compound consumption

The compound was administered in gelatin capsules every morning before feeding, the amount given being determined by the most recent body weight measurement (see Table 1).

3. Food efficiency

Food efficiency was not calculated by the study author, but was calculated by the reviewer because there was some effect on body weight gain in both sexes of animals. The results shown in Table 4 indicate that there was a tendency toward lower food efficiencies with increased dose. The lowered overall food efficiencies at 250 mg/kg/day in both sexes may be partly due to the elevated incidence of fluid feces (and fluid loss) at this dose.

TABLE 4: Food efficiency in dogs given ICIA5504 for 92 or 93 days[1]

Dose (mg/kg/day)	Males	Females
0	8.65	6.49
10	7.30 (-16%)	4.63 (-29%)
50	7.36 (-15%)	4.32 (-33%)
250	5.77 (-33%)	4.03 (-38%)

Data taken from pages 43-45, MRID 43678136.
[1] Values were calculated by the reviewer. The decrease in food efficiency relative to control values is enclosed in the parenthesis.

D. OPHTHALMOSCOPIC EXAMINATION

No treatment-related effects were observed.

E. BLOOD WORK

1. Hematology

There were statistically significant differences from controls for several parameters in both male and female dogs. At 250 mg ICIA5504/kg/day, there was a small decrease (i.e. < 5%, $p \leq 0.05$ or 0.01) relative to controls in males in the mean cell volume (MCV) (weeks 8,13), in males (weeks 4, 8, 13) and females (weeks 8, 13) in the mean cell hemoglobin (MCH), and in males in the mean cell hemoglobin concentration (MCHC) (week 13). The MCH was also slightly lower (2.6%, $p \leq 0.05$) than controls in 50 mg/kg/day males at week 13. Platelet counts were increased by 16-26% ($p \leq 0.05$ or 0.01) in males at 250 mg/kg/day (weeks 4,13) and in females at both 50 and 250 mg/kg/day (weeks 4,8,13) compared to concurrent controls. The platelet counts of females were within 9% of pre-experimental values, and there was no clear dose-response in either sex. Due to the small magnitude of all the hematologic alterations and/or their lack of dose-response, they are not considered biologically or toxicologically significant.

2. Clinical Chemistry

Statistically significant alterations compared to concurrent controls ($p \leq 0.05$ or 0.01) were seen during one or more weeks in numerous parameters. In high-dose dogs of both sexes, there were moderate increases in the levels of plasma cholesterol (13-26%; males weeks 4,8; females week 13), triglycerides (42-89%; males week 4; females, all weeks), and alkaline phosphatase (24-87%; both sexes all weeks) and a small decrease in plasma albumin (7.9-11.6%, both sexes all weeks). Mid-dose females had elevated plasma alanine transaminase (101-144%, weeks 4,13). Cholesterol was elevated 17-25% in low-dose (weeks 8, 13) and mid-dose males (weeks 4, 8, 13). These results are summarized in Table 5, and suggest there was an adverse affect on liver function and/or biliary function and flow from compound treatment. The lack of a consistent increase in the severity of the clinical effect with time and/or dose in some cases, as well as the lack of histopathological correlates indicates the changes were an adaptive liver response to treatment. Other parameters altered at one or more weeks ($p \leq 0.05$ or 0.01) were incidental to treatment because they were very small, transient, and/or lacked dose-response: altered plasma sodium (1.3% decrease in high-dose males, week 4; 1.6% increase in mid-dose females, week 8), creatinine (11% increase in high-dose males, week

8; 10-12% decrease in mid or high-dose females, week 4), and total protein (5% decrease in high-dose males, week 13; 6.4% increase in low-dose females, week 4).

TABLE 5: Clinical chemistry changes in dogs given ICIA5504[1]

Parameter	Week	Dose (mg/kg/day)			
		0	10	50	250
Males					
Albumin (g/100 Ml)	-1	2.95	2.98	3.03	3.03
	4	3.03	2.99	2.99	2.79**
	8	2.98	2.88	2.87	2.70**
	13	3.10	3.10	3.03	2.80**
Cholesterol (mg/100 Ml)	-1	177	149	157	133
	4	137	156	171**	166*
	8	154	181*	188**	183*
	13	143	167*	176**	161
Triglycerides (mg/100 Ml)	-1	34.5	29.8	32.0	31.3
	4	28.9	34.9	31.1	41.1*
	8	26.4	24.5	23.4	33.0
	13	22.1	24.0	28.2	33.1
Alkaline phosphatase (IU/L)	-1	200	185	213	191
	4	162	181	192	210**
	8	142	171	179	203**
	13	122	161	167	228**
Females					
Albumin (g/100 Ml)	-1	3.10	3.13	3.15	3.10
	4	3.25	3.17	3.14	2.93**
	8	3.14	3.07	3.00	2.84**
	13	3.28	3.27	3.07	2.90**
Cholesterol (mg/100 Ml)	-1	166	183	150	167
	4	154	167	163	167
	8	168	171	176	185
	13	157	174	174	198**
Triglycerides (mg/100 Ml)	-1	37.3	28.3	24.8	27.8
	4	22.8	28.2	30.5	38.0**
	8	24.5	26.0	30.0	38.2**
	13	18.8	23.6	28.1	35.5*
Alkaline phosphatase (IU/L)	-1	212	208	198	204
	4	181	191	193	224*
	8	166	169	178	215*
	13	158	163	156	234*
Plasma alanine transaminase (IU/L)	-1	15.8	15.3	15.0	17.8
	4	17.7	23.1	35.6*	25.9
	8	18.8	21.9	31.7	19.3
	13	19.6	22.0	47.8*	26.9

Data taken from pages 60-77, MRID 43678136.
[1]The values presented for treatment weeks 4, 8, and 13 are adjusted means that were obtained by making a covariate adjustment based on the separate sex pre-experimental group means. [The pre-treatment values (week -1) were not adjusted.]
*Statistically different from control group mean at the 5% level
**Statistically different from control group mean at the 1% level

F. URINALYSIS

Significant differences from controls were not found for any quantitative or qualitative tests.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

There were no statistically significant alterations in absolute or relative to body organ weights at any dose in males; in all cases organ weights were within 10% of controls and/or changes were not dose-related. In females, there was a small, dose-related increase in the absolute liver weight at 50 mg/kg/day (6.3%, $p \leq 0.05$) and at 250 mg/kg/day (9.3%, $p \leq 0.01$), though the relative to body weight increases were not statistically significant. The thyroid weight of the 250 mg/kg/day females was also elevated (37%, $p \leq 0.01$) when compared to a control group of only 3 animals--one was excluded due to chronic thyroiditis (and enlarged thyroids). Relative thyroid weights were largely unaffected. The statistically significant organ weight changes are presented below in Table 6. It is unclear whether the increases in liver and thyroid weights in females were treatment-related. The absence of histopathologic correlates indicates that the organ weight changes may not be toxicologically significant. [Note: Because there were no differential effects on the left and right paired organs, the data for combined weights only were statistically analyzed and tabulated by the study author.]

TABLE 6: Organ weight changes in female dogs given ICIA5504

Organ	Dose (mg/kg/day)			
	0	10	50	250
Terminal body weight(g)	12925	12375	12175	11850
Liver Absolute weight Organ/body weight ratio(%)	367 2.85	371 2.99	390* 3.22	401** 3.41
Terminal body weight (g)	12800[1]	12375	12175	11850
Thyroid Absolute weight Organ/body weight ratio(%)	0.70 0.006	0.86 0.007	0.83 0.007	0.96** 0.008

Data taken from pages 85-92, MRID 43678136.

*Statistically different from control group mean at the 5% level;
**Statistically different from control group mean at the 1% level
[1]The terminal body weight of one of the four control females was excluded in calculating the group mean thyroid weight because the female had chronic thyroiditis and enlarged thyroids.

2. Gross pathology

There were no significant macroscopic alterations in either sex of dogs. Lesions were seen in one or two animals/sex in the brain (dilated ventricles), eye (red area), liver (lobular pattern, pale area), lungs (color and/or texture changes), pituitary gland (cyst), skin (mass), and urinary bladder (mucosa red areas/spots). The low incidence of the macroscopic lesions indicated that they were spurious and not toxicologically relevant.

3. Microscopic pathology

a) Non-neoplastic - Neither the incidence or severity of any microscopic lesions was significantly elevated compared to controls. The lesions seen in numerous organs in both male and female dogs are ones commonly occurring in Alderley Park beagles of this age.

b) Neoplastic - There was no evidence that compound treatment caused a neoplastic response in any group of animals. A neoplastic lesion was found in only one animal--a high-dose female--which was histologically determined to be a benign primary histiocytoma.

III. DISCUSSION

A. DISCUSSION

Groups of four male and four female beagle dogs were given ICIA5504 in gelatin capsules at doses 0, 10, 50, or 250 mg/kg/day for 92 or 93 days (one half of each group having 92 and one half 93 treatment days). Controls received empty gelatin capsules. Water was provided *ad libitum* and 350 g or 400 g food per female or male, respectively, was provided daily. The dogs were examined twice daily for clinical signs of toxicity and weighed weekly. Hematological and clinical chemistry parameters were measured at week -1 and during treatment weeks 4, 8, and 13.

No animals died during this study. The most notable treatment-related clinical signs were salivation at dosing, fluid feces, and minor increases in salivation, vomiting, and regurgitation in both sexes throughout the treatment period (statistical analysis was not performed). The relationship between compound treatment and the clinical signs is not known; they suggest a neurological effect or intolerance of the compound (e.g. gastrointestinal irritation). There was no evidence to support either possibility. The effects on body weight and weight gain were generally dose-related, being most marked at 250 mg/kg/day in both males and females. The weight changes were relatively small: all weekly weights were within 9% of controls ($p \leq 0.05$ or 0.01) and the overall weight gain was 1/3 lower for both sexes. There was no dose-related decrease in food consumption and only a minor decrease in food efficiency at the highest dose in both sexes. The weight decreases and lowered food efficiency may have ben partly due to the increased vomiting, regurgitation, and loose stool in the animals. Several hematology parameters were statistical significantly different from controls ($p \leq 0.05$ or 0.01) in one or both sexes primarily at the high dose. They were neither biologically nor toxicologically significant because they were within 5% of controls (MCV, MCH, and MCHC) or not dose-related and similar to pre-treatment levels (platelet counts).

A number of clinical chemistry alterations occurred in both sexes at the high dose that are consistent with an effect on liver and possibly biliary function. Changes included increased plasma cholesterol (13-26%), triglycerides (42-89%), and alkaline phosphatase (24-87%), and lowered plasma albumin (7.9-11.6%). Additionally, alanine transaminase was increased (101-144%) in mid-dose females and cholesterol was increased mid- and low-dose males (17-25%). These changes were accompanied by increased liver weight in mid- and high-dose females (6.3%, $p \leq 0.05$; 9.3%, $p \leq 0.01$, respectively). Both the liver weight changes and the clinical chemistry effects were relatively minor. The absence of definitive dose and temporal relationships for the noted minor changes in the liver, and the lack of histopathologic correlates suggest that the observed effects were an adaptive response of the liver to xenobiotic exposure. Changes in several other clinical chemistry parameters were not clearly treatment-related (plasma sodium, creatinine, and total protein).

No treatment-related effects were seen in either sex on ophthalmology, urinalysis, or gross or microscopic pathology. The increase in only the absolute thyroid

weight of high-dose females (37%, $p \leq 0.01$) was of unknown toxicological significance; there were no histopathological correlates. Based on an increased occurrence of several clinical signs and minor weight decreases and clinical chemistry changes, the LOAEL (lowest observable adverse effect level) is 50 mg/kg/day for both sexes of dogs under the conditions of this study. The NOEL for both male and female dogs is 10 mg/kg/day due to a lack of toxic effects.

B. STUDY DEFICIENCIES

There were no major deficiencies that would impact the interpretation or classification of this study. The minor deficiencies include failure to statistically analyze the net body weight gains and clinical observations, to calculate the food efficiencies, and to weigh the parathyroid glands. Although it was not required, it would have been helpful if the author had provided the rationale for the selected test doses, and if the level of plasma glutamate dehydrogenase were measured since there were indications that the compound affected liver function.