008452

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy 6/27/91
Section IV, Tox. Branch II, (H7509C)

Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 7/1/91
Section IV, Tox. Branch II, (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Chronic Oral Toxicity - Dog (§83-1)

TEST MATERIAL: R-4572 Tox. Chem. No.: 444

SYNONYMS: ORDRAM, MOLINATE MRID No.: 417811-01

STUDY NUMBER: T-13236

SPONSOR: ICI Americas Inc.
Wilmington, DE

TESTING FACILITY: Ciba-Geigy Corporation
Environmental Health Center
400 Farmington Avenue
Farmington, CT 06032

TITLE OF REPORT: One-Year Toxicity Study with R-4572 in Beagle Dogs

AUTHORS: John C. Pettersen and Peter F. Wadsworth

REPORT ISSUED: December 17, 1990

CONCLUSIONS:

R-4572 administered orally by gelatin capsule at doses of 0, 1, 10, 50 and 100 mg/kg/day daily for one year to beagle dogs, caused the following adverse effects at 50 mg/kg/day and 100 mg/kg/day (dosed only for 14 weeks, then empty capsules): various effects on the nervous systems, a decrease in body weight gain, anemia and a decrease in ejaculate volume as well as the percent of motile sperm.

The Maximum Tolerated Dose (MTD) was considered to have been achieved: 50 mg/kg/day

The No Observed Effect Level (NOEL) = 10 mg/kg/day

The Lowest Observed Effect Level (LOEL) = 50 mg/kg/day (statistically significant decreased body weight gain, adverse effects on the nervous systems, anemia and decreased ejaculate plus percent of mobile sperm)

Classification: Core Supplementary - This may be upgraded if the Registrant provides acceptable data regarding test article purity and stability.

This study does not satisfy the Guideline Requirements (§83-1) for a chronic oral toxicity study in dogs.

~~33~~

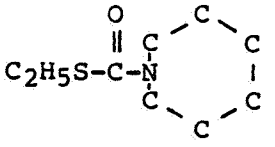
008452

I. Materials, Methods and Results

A. Test Article Description

Name: S-Ethyl Hexahydro-1H-Azepine-1-Carbothioate

Formula:



Lot Number: WRC-492108-22, EHC code No. 0866-30
Purity: 97.6 % (w/w)
Physical Property: liquid
Stability: stable at room temperature

B. Test Article Analyses for Purity and Stability

Report page 13, MATERIALS AND METHODS, 3.2 Test Substance Data states: "The sample tested was characterized and results obtained were filed with other study data."

This Reviewer was unable to locate in this report analyses data refering to test article purity and stability. As the same lot number was used during the one-year study, data need to show that the 97.6% purity was present at the end of 12 months of dosing as well as at study initiation.

NOTE: Unless the Registrant provides purity and stability analytical data, this study can only be accepted as CORE SUPPLEMENTARY and therefore would not fulfill Guideline §83-1. Analytical data acceptable to the Agency could allow the study to be upgraded to CORE MINIMUM.

C. Animals

Male and female (22 of each) beagle dogs, four to five months of age, were received from Marshall Farms, North Rose, NY and were assigned to this study. During an approximate 34 day quarantine period the dogs were observed daily, exercised weekly and examined by a veterinarian (including clinical laboratory tests).

Four to six days prior to study initiation, the animals were given pretest physical examinations, weighed and assigned to study groups (4/sex/group) so that group mean body weights were similar. Body weights at the time of treatment initiation were

6.8-9.9 kg for males and 5.4-7.9 kg for females. Room temperatures and humidity were maintained between 19 and 24°C and 40 and 60%, respectively. There was a 12 hour light/dark cycle. The dogs received 350 g/day of Purina Certified Canine Diet #5007 and tap water ad libitum (automatic watering system).

D. Dosing

The dogs were administered the test article daily in 000 gelatin capsules. Dose levels were 0 (control, empty capsule), 1, 10, 50 and 100 mg/kg/day. Dosing of the 100 mg/kg/day group was discontinued after study week 14 because of severe toxicity (dogs received empty capsules for the remainder of the study). The amount of R-4572 given each dog was calculated weekly and was based on the animal's most recent body weight. Capsules were administered usually in the late morning, 2-3 hours post-feeding.

Dose levels were selected based on the results of a 4-week range-finding study, T-13233, (mg/kg/day): 150, highest dose tested = "severe toxicity" (not defined in one-year study report; 100 = "definite toxicity" (emesis, ataxia); 75 = minimal toxicity (not defined in the one-year study report); 25 = "no signs of toxicity".

E. General Observations

1. Mortality and Clinical Signs - All dogs were observed at least twice daily.

There was no mortality.

Clinical observations which appeared to be the result of test article administration are presented in Table 1.

Table 1

APPARENT R-4572 RELATED CLINICAL OBSERVATIONS IN DOGS TREATED FOR ONE YEAR†

	Males ††					Females ††				
mg/kg/day =	0	1	10	50	100	0	1	10	50	100
Ataxia	0a	0	0	4	4	0	0	0	4	4
Cyanosis	0	0	0	0	2	0	0	0	0	0
Dyspnea	0	0	0	1	4	0	0	0	0	2
Pallor, general	0	0	0	1	3	0	0	0	1	0
Reduced locomotor activity	0	0	0	4	4	0	0	0	4	4
Splayed hind limbs	0	0	0	4	4	0	0	0	4	4
Tremors	0	0	1	4	4	0	0	1	4	4

† = Dogs received 100 mg/kg/day for 14 weeks, then empty capsules.
†† = 4 dogs/sex/group
a = number of dogs with observations
Data extracted from report Tables 1 and 2, pages 39-50.

31

3

R-4572 had a pronounced effect on nervous system activity at 100 (during 14 weeks of dosing) and 50 mg/kg/day in males and females. In addition to the data shown in Table 1, there were numerous episodes of emesis and salivation in the 100 mg/kg/day dogs, sporadic episodes of salivation in the 10 and 50 mg/kg/day males and a high incidence of salivation in the 50 mg/kg/day females.

In the 100 mg/kg/day group, 6/8 dogs appeared to be unable to bark or had an attenuated bark intensity during the second and third months. By months 7 or 8, 4/6 affected animals seemed to have regained their ability to bark. Of the eight dogs receiving 50 mg/kg/day, five were reported to have lost the ability to bark or had an attenuated bark at about the seventh-eighth months.

2. Neurological Signs - Neurological examinations were performed by a board certified specialist in veterinary neurology (Kenneth L. Schunk, DVM - letters of neurological evaluations on report pages 310-314) and were conducted pretest as well as at 3, 6, 9 and 12 months. [Details of procedures were described in Appendix D, pages 243 and 244.] A summary of neurologic examinations is presented in Table 2 (a composite of report Tables 9a and 10a, pages 68 and 76).

   The following neurological parameters were examined (report Tables 9B and 10B, pages 69-75 and 77-83):

   Hopping, forelimbs & hindlimbs
   Hemistanding & Hemiwalking, left & right
   Extensor Postural Thrust
   Proprioceptive Positioning, forelimbs & hindlimbs
   Wheelbarrowing, forelimbs
   Patellar Reflex, left & right
   Ankle Jerk Reflex, left & right
   Biceps Reflex, left & right
   Triceps Reflex, left & right
   Flexor Reflex, forelimbs & hindlimbs, left & right

Test article related neurological changes were noted at all test intervals but only in dogs treated with 50 and 100 mg/kg/day. The severity increased in the 50 mg/kg/day dogs as the study progressed and essentially remained the same in the 100 mg/kg/day-recovery animals. The findings were more severe at 100 than at 50 mg/kg/day. Males appeared to be more severely affected than females.

Findings included the following (increased incidence of dogs exhibiting the observation):

Depressed postural reactions (forelimb and/or hindlimb hopping)
Depressed hemistanding and hemiwalking
Depressed extensor postural thrust

008452

Table 2

A SUMMARY OF NEUROLOGICAL EXAMINATION RESULTS - DOGS TREATED WITH R-4572 FOR ONE YEAR

		Males					Females				
mg/kg/day =		0	1	10	50	100	0	1	10	50	100
MEASUREMENT	MONTH										
Mental attitude/ behavior	0	0/4a	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	3	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	6	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	9	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	12	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Gait and posture	0	4/4	4/4	3/4	4/4	3/4	4/4	4/4	4/4	4/4	3/4
	3	3/4	3/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
	6	2/4	4/4	4/4	4/4	4/4	3/4	4/4	4/4	4/4	4/4
	9	1/4	3/4	4/4	4/4	4/4	1/4	2/4	4/4	4/4	4/4
	12	1/4	2/4	4/4	4/4	4/4	1/4	2/4	2/4	4/4	4/4
Postural reactions	0	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	3	0/4	0/4	0/4	4/4	4/4	0/4	0/4	0/4	2/4	4/4
	6	0/4	0/4	0/4	4/4	4/4	0/4	0/4	0/4	4/4	4/4
	9	0/4	0/4	0/4	3/4	4/4	0/4	0/4	0/4	4/4	4/4
	12	0/4	0/4	0/4	3/4	4/4	0/4	0/4	0/4	2/4	4/4
Spinal reflexes	0	2/4	1/4	2/4	3/4	1/4	2/4	1/4	0/4	1/4	1/4
	3	1/4	1/4	1/4	2/4	0/4	0/4	0/4	1/4	1/4	1/4
	6	2/4	1/4	1/4	4/4	3/4	1/4	0/4	0/4	2/4	1/4
	9	0/4	0/4	0/4	2/4	2/4	1/4	0/4	1/4	3/4	2/4
	12	2/4	1/4	1/4	4/4	4/4	1/4	1/4	1/4	3/4	4/4
Sensation	0	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	3	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	6	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	9	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	12	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Muscle tone	0	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	3	0/4	0/4	0/4	3/4	3/4	0/4	0/4	0/4	2/4	3/4
	6	0/4	0/4	0/4	4/4	3/4	0/4	0/4	0/4	2/4	4/4
	9	0/4	0/4	0/4	2/4	3/4	0/4	0/4	0/4	0/4	0/4
	12	0/4	0/4	0/4	3/4	3/4	0/4	0/4	0/4	0/4	2/4
Cranial nerves	0	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	3	0/4	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4
	6	0/4	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4
	9	0/4	0/4	0/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4
	12	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	1/4

a = Number of dogs showing anything other than normal response/number of dogs examined.

Data extracted from Report Tables 9a and 10a, pages 68 and 76.

~~33~~

5

Depressed propriceptive positioning
Depressed forelimb wheelbarrowing
Hyperreflexic patellar reflexes
Gait abnormalities (awkward hopping, ataxia, awkward gait, stiff/stilted gait, mistakes, dysmetric gait and sliding of rear legs)

Decreased muscle mass/atrophy was observed as follows:

Table 3

OBSERVATION OF MUSCLE MASS/ATROPHY IN DOGS ADMINISTERED R-4572

	Males†				Females†			
mg/kg/day \ months =	3	6	9	12	3	6	9	12
50	0	3	0	0	0	1	0	0
100	3	3	1	1	0	0	0	0

† = 4 dogs/sex/group
Data extracted from report page 26 and Tables D1 and D2, pages 245-309.

3. Body Weights - All animals were weighed pre-dosing and weekly thereafter.

Table 4 presents selected group mean body weights and body weight gains.

In 50 and 100 mg/kg/day males, there was a statistically significant ($p<0.05$ or 0.01) lower body weight compared with control values from about the end of the second month of the study until termination. The 100 mg/kg/day dogs were taken off test article at week 14 and (as noted in Table 4), from week 16 until week 52, they gained 3.0 kg compared with a control gain of 2.1 kg. This would indicate a "recovery". The 50 mg/kg/day dogs gained 1.2 kg from week 16 until week 52 compared with the control group gain.

In females (at the weighing intervals presented in Table 4) administered 100 mg/kg/day, there were no statistically significant decreases in body weight compared with control values and at 50 mg/kg/day, only three intervals ($p<0.05$). After removal from 100 mg/kg/day of test article (week 14), females gained a group mean of 2.4 kg from weeks 16-52 compared with 1.9 kg for controls, and the 50 mg/kg/day group gained 1.0 kg compared with the control group gain. This also would indicate a "recovery" for the 100 mg/kg/day dogs.

Table 4

BODY WEIGHTS, WEIGHT GAINS AND PERCENT FROM CONTROL FOR DOGS ADMINISTERED R-4572 BY CAPSULE FOR ONE YEAR

	Males†					Females†				
mg/kg/day =	0	1	10	50	100	0	1	10	50	100
WEEKS ON TEST	GROUP MEAN BODY WEIGHTS (kg)									
0	8.4	8.3	8.1	7.9	7.9	7.0	6.4	6.8	6.4	6.5
1	8.8	8.8	8.5	8.4	8.3	7.1	6.6	7.3	6.8	6.7
2	9.1	9.0	8.7	8.4	8.3	7.5	6.8	7.4	7.0	7.0
3	9.5	9.3	9.0	8.8	8.5	7.8	7.1	7.7	7.4	7.4
4	10.0	9.8	9.7	9.0	8.6	8.4	7.5	7.9	7.8	7.6
8	11.0	10.2	10.1	9.3	8.3**	9.3	7.8	8.4	7.8	7.7
12	12.1	11.1	11.1	9.9*	8.6**	9.9	8.6	8.6	8.6	8.3
16	12.5	11.4	11.3	9.6**	8.3**	10.4	8.6	8.9	8.3	8.1
20	13.0	11.7	11.6	10.1*	8.9**	10.7	9.0	9.0	8.8	8.8
24	13.4	11.9	11.8	10.5*	9.4**	10.9	9.2	9.3	8.9	9.1
28	13.7	12.1	12.1	10.4*	10.0**	11.3	9.2	9.4	9.0	9.5
32	13.7	12.1	11.8	10.3*	10.5*	11.1	9.3	9.4	9.0	9.8
36	14.0	12.1	11.8	10.7*	11.0*	11.9	9.7	9.5	9.0*	9.9
40	14.3	12.1	11.9	10.6*	10.9*	12.0	9.4*	10.5	9.8	10.6
44	14.5	12.3	12.3	10.9*	11.3	12.1	9.7	9.6	9.1*	10.2
48	14.9	12.5	12.5	11.1*	11.6	12.3	9.9	10.0	9.2*	10.7
52	14.6	12.3	12.2	10.8*	11.3*	12.3	9.8	9.7	9.3	10.5
WEEKS	GROUP MEAN BODY WEIGHT GAIN (kg)									
0-4	1.6	1.5	1.6	1.1	0.7	1.4	1.1	1.1	1.4	1.1
0-8	2.6	1.9	2.0	1.4	0.4	2.3	1.4	1.6	1.4	1.2
0-12	3.7	2.8	3.0	2.0	0.7	2.9	2.2	1.8	2.2	1.8
0-24	5.0	3.6	3.7	2.6	1.5	3.9	2.8	2.5	2.5	2.6
0-36	5.6	3.8	3.7	2.8	3.1	4.9	3.3	2.7	2.6	3.4
0-52	6.2	5.0	4.1	2.9	3.4	5.3	3.4	2.9	2.9	4.0

† = 4 dogs/sex/group
Statistical Significance: $p<0.05$ = *; <0.01 = **

Data extracted from (or calculated from) Report Table 3, pages 51-62.

4. Food Consumption - Food consumption was measured weekly during the first 3 months of the study and then monthly thereafter (each dog was given 350 g of food/day).

There was a similar amount of food consumed by each group throughout the year study with the exception of the 100 mg/kg/day dogs which ate less than the control group during weeks 4 through 11.

5. Ophthalmoscopic Examinations - All dogs were examined prior to the start of dosing and at the termination of the study.

No ophthalmic abnormalities were reported.

F. Clinical Pathology

Hematology, blood chemistry and urinalysis parameters from each animal were examined pre-test (twice for hematology and clinical chemistry) as well as at 3, 6 and 12 months. Dogs were fasted overnight prior to sample collection (urine and feces collected in metabolism cages). Blood was obtained from a jugular vein.

1. Hematology - The CHECKED (x) parameters were examined.

X		X	
x	Hematocrit (HCT)*	-	Total plasma protein (TP)
x	Hemoglogin (HBG)*	x	Leukocyte differential count
x	Leukocyte count (WBC)*	x	Mean corpuscular HGB (MCH)
x	Erythrocyte count (RBC)*	x	Mean corpuscular HGB conc. (MCHC)
x	Platelet count*	x	Mean corpuscular volume (MCV)

x Reticulocyte count - determined when hematocrit <32.0% at pre-test, 3 and 6 months intervals and on all dogs at 12 months
x RBC osmotic fragility (12 months)
x Prothrombin Time (PT)
x Activated partial thromboplastin (aPTT)

* = EPA Guideline Requirement "-" = Not examined

Table 5 presents those hematological parameters which were possibly altered by test article administration.

Though not statistically significant ($p<0.05$ or 0.01) in all instances, data presented in Table 5 show a degree of anemia (decreased erythrocytes, hemoglobin and hematocrit compared with control values) in the 50 and possibly 10 mg/kg/day male groups. Dogs administered 100 mg/kg/day also showed the decreases in these parameters, but only at the 3-month interval with recovery

Table 5

HEMATOLOGICAL PARAMETERS POSSIBLY ALTERED BY THE ADMINISTRATION OF R-4572 TO DOGS FOR ONE YEAR

mg/kg/day =	Males†					Females†				
	0	1	10	50	100	0	1	10	50	100
MONTH	RED BLOOD CELL (mil/cu mm)									
3	7.3	6.9	6.6	6.2*	5.4**	7.3	6.8	7.0	6.1	5.7**
6	7.3	7.0	6.7	6.3	6.8	7.4	6.6	7.2	6.3	7.0
12	7.1	7.2	7.0	6.3	7.2	7.4	7.3	7.3	6.3	7.0
	HEMOGLOBIN (G/DL)									
3	16.8	15.9	14.9	14.6*	13.0**	16.6	15.8	16.2	14.6	13.3**
6	17.4	16.3	15.7	15.1	16.2	17.3	15.8	16.8	15.1	16.2
12	17.0	16.6	15.8	15.0	16.8	17.4	17.3	17.2	14.9	16.3
	HEMATOCRIT (%)									
3	46.6	44.1	40.4*	39.7*	34.6**	44.9	43.2	44.4	39.0	35.8**
6	48.2	45.4	42.2*	40.7*	44.8	48.6	43.8	47.2	41.6	45.9
12	44.3	44.4	42.2	39.3	44.6	46.1	46.3	45.2	39.3	44.5
	PLATELET (thous/cu mm)									
3	350	359	395	528*	489	383	415	368	491	490
6	297	338	343	474**	379	372	410	382	528**	394
12	308	318	352	499**	379	358	461	430	580**	375
	RBC OSMOTIC FRAGILITY (EC50)									
12	0.44	0.44	0.42	0.39**	0.44	0.46	0.45	0.44	0.40**	0.49

† = 4 dogs/sex/group

Statistical Significance: $p<0.05$ = *; <0.01 = **

Data extracted from Report Tables 12 and 13, pages 85-99.

apparent at the 6 and 12 month intervals (ceased to receive test article after week 14). A significant ($p<0.05$ or 0.01) increase in platelets was reported in 50 mg/kg/day males at 3, 6 and 12 months with a non-significant increase in 100 mg/kg/day at the 3-month interval only.

In females, though not statistically significant, erythrocyte, hemoglobin and hematocrit values were suggestively below controls in dogs administered 50 mg/kg/day at all three intervals. The 100 mg/kg/day females showed lower ($p<0.01$) values for these parameters at the 3-month interval only. Platelet counts were elevated ($p<0.01$) in 50 mg/kg/day females at 6 and 12 months.

Red blood cell osmotic fragility appeared to be greater ($p<0.01$) than controls in the 50 mg/kg/day dogs of both sexes.

2. Blood Chemistry - The CHECKED (X) parameters were examined

X	Electrolytes
x	Calcium*
x	Chloride*
-	Magnesium*
x	Phosphorous*
x	Potassium*
x	Sodium

X	Other
x	Albumin*
x	Blood creatinine*
x	Blood urea nitrogen*
x	Cholesterol*
x	Globulin
x	Glucose*
x	Total Bilirubin*
x	Total Protein*
x	Triglycerides

	Enzymes
x	Alkaline phosphatase
x	Cholinesterase (serum, RBC and brain)
x	Creatinine phosphokinase*
-	Lactic acid dehydrogenase
x	Serum alanine aminotransferase (also SGPT)*
x	Serum aspartate aminotransferase (also SGOT)
x	Gamma glutamyl transferase
x	Direct bilirubin (when total bilirubin >0.4 mg/dl)
x	Sorbitol deghydrogenase
x	Albumin/globulin ratio

* = EPA Guideline Requirement "-" = Not examined

Table 6 presents clinical chemistry parameters possibly altered by the administration of R-4572.

Serum cholesterol was significantly ($p<0.01$) increased in 50 mg/kg/day males at 3, 6 and 12 months as well as in 100 mg/kg/day males at 3 months only. Females at these doses and intervals showed a similar non-significant pattern.

Table 6

CLINICAL CHEMISTRY PARAMETERS POSSIBLY ALTERED BY THE ADMINISTRATION OF R-4572 TO DOGS FOR ONE YEAR

	Males†				
mg/kg/day =	0	1	10	50	100
Serum Cholesterol (mg/dl)					
3 months	145	142	160	244**	234**
6 months	153	152	171	249**	165
12 months	151	145	172	234**	159
Alkaline Phosphatase (I.U./L)					
3	125	135	134	160	209
6	69	92	82	136*	133
12	58	70	64	127**	89
Serum Cholinesterase (I.U./L)					
3	2107	1968	1857	1669	1880
6	2152	2075	1919	1562	2099
12	2117	2282	2128	1574	2162

	Females†				
mg/kg/day =	0	1	10	50	100
Serum Cholesterol (mg/dl)					
3	157	132	161	242*	207
6	229	163	186	282	214
12	204	161	174	263	245
Alkaline Phosphatase (I.U./L)					
3	154	147	118	193	258
6	113	92	88	199	176
12	100	99	86	253	147
Serum Cholinesterase (I.U./L)					
3	2161	1848	2204	1576**	1738**
6	2240	1872	2200	1645*	2485
12	2213	2011	2377	1627*	2436

† = 4 dogs/sex/group

Statistical Significance: $p<0.05$ = *; <0.01 = **

Data extracted from Report Tables 14 and 15, pages 100-115.

Alkaline phosphatase levels appeared to be increased ($p<0.05$, $p<0.01$ or not significant) in males and females at 50 mg/kg/day at 3, 6 and 12 months and at 100 mg/kg/day at 3 months and possibly at 6 and/or 12 months.

Serum cholinesterase values were lower than controls in 50 mg/kg/day males (not significant) and females (significant, $p<0.05$ or 0.01) at 3, 6 and 12 months. Males and females administered 100 mg/kg/day showed some (not significant in males, $p<0.01$ in females) decrease at 3 months only.

3. Urinalysis - The CHECKED (X) parameters were examined.

X		X	
x	Appearance*	x	Glucose*
x	Volume*	x	Ketones*
x	Specific gravity	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*	-	Nitrate
x	Protein*	x	Urobilinogen

* = EPA Guideline Requirement "-" = Not examined

There were no apparent test article induced changes in any of the parameters examined.

4. Sperm Analysis - Sperm collection and analysis were performed after 6 months and near the end of the study. Analyses were carried out because R-4572 is known to have produced a rodent specific effect on sperm and the purpose in this study was to determine if the test article had effects on dog sperm.

First and second ejaculates were collected in graduated collection tubes by manual stimulation. A portion of the ejaculate was diluted with Ham's F-10 nutrient mixture with L-Glutamine fortified with 10 mg/ml BSA Fraction V prior to assessment of sperm motility and concentration. About 200 sperm were counted and scored motile/non-motile (hemocytometer). Sperm concentration was determined by counting the total number of sperm in four squares of the hemocytometer grid and was expressed as millions/ml and millions/ejaculate. The number of live /dead sperm and morphology were evaluated using an eosin-migrosin staining procedure. About 200 sperm were counted and classified as live or dead and 200 were examined for morphology. [See Table 7.]

Table 7

SUMMARY OF SPERM ANALYSIS DATA FROM DOGS ADMINISTERED R-4572 FOR ONE YEAR

Dose Group (mg/kg/day)	Sample Vol. ml	% Motile	Million per ml	Million/ sample	% abnormal	% Live
6 Month Interval						
0	1.43	62	922	1476	21	66
1	1.90	78*	1508	3323	10	73
10	1.35	51	811	1002	13	70
50	1.08	47	903	1053	25	67
100	0.95	49	641	466	32	50
12 Month Interval						
0	1.23	85	122	163	9	67
1	1.83	76	72	128	10	61
10	0.78	64	279	201	12	73
50	0.38*	44*	322	117	20	58
100	0.95	79	134	108	7	85

NOTE: 4 dog/group

Statistical Significance: $p<0.05$ = *

Data extracted from Report Table 8, pages 66 and 67.

Although the only statistically significant ($p<0.05$) negative effects observed were a decrease in ejaculate sample volume and percent motility in the 50 mg/kg/day dogs at 12 months, there appeared to be a decrease (not statistically significant) in volume in the 100 mg/kg/day group at 6 months.

G. Sacrifice and Pathology

All dogs were sacrificed and necropsied after an overnight fast at the end of the study (days 371-375). The animals were anesthetized with sodium pentobarbital (i.v.) and euthanitized by exsanguination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs were weighed.

X	Digestive system
-	Tongue
x	Salivary glands*
x	Esophagus*
x	Stomach*
x	Duodenum*
x	Jejunum*
x	Ileum*
x	Cecum*
x	Colon*
x	Rectum*
xx	Liver*
x	Gallbladder*
x	Pancreas*

X	Respiratory
x	Trachea*
x	Lung*
	Cardiovasc./Hemat.
x	Aorta*
xx	Heart*
x	Bone marrow*
x	Lymph nodes*
x	Spleen*
x	Thymus*

X	Urogenital
xx	Kidneys*
x	Urinary bladder*
xx	Testes*
x	Epididymides*
x	Prostate*
-	Seminal vesicle*
xx	Ovaries*
x	Uterus*

X	Neurologic
xx	Brain*
x	Peripheral Nerve*
x	Spinal cord (3 levels)
xx	Pituitary*
x	Eyes (optic nerve)*

X	Glandular
xx	Adrenals*
-	Lacrimal gland
x	Mammary gland*
x	Parathyroids*
xx	Thyroids*

X	Other
x	Bone*
x	Skeletal muscle*
x	Skin*
x	All gross lesions and masses*

x Larynx x Nasal passage x Vagina

* = EPA Guideline Requirement "-" = Not examined

1. Macroscopic

There were no findings which were considered related to test article administration.

2. Organ Weights

The following organs were weighed and the weights expressed as absolute (g) and relative (organ weight/body weight ratio and organ weight/brain weight ratio - %): adrenals, brain, heart, kidneys, liver, pituitary, testes and thyroids (with parathyroids).

Table 8 presents those absolute and/or relative organ weights which were either statistically or appeared to be possibly biologically different from control values.

Absolute and relative (to body and brain) adrenal weights of male and female 50 mg/kg/day dogs were increased (statistically or not statistically) over control values.

~~43~~

14

Table 8

FINAL BODY AND SELECTED ORGAN WEIGHTS OF DOGS ADMINISTERED R-4572 FOR ONE YEAR

	Males†					Females†				
mg/kg/day =	0	1	10	50	100	0	1	10	50	100
Final body wt. (kg)	14.8	12.3	12.3	10.8	11.1	12.1	9.6	9.6	9.2	10.3
% from control	-	17	17	27	25	-	21	22	25	15
Adrenals (g)	1.25	1.29	1.42	1.65	1.19	1.45	1.40	1.40	1.91	1.60
% body wt.	.009	.011	.012	.016	.011	.012	.015	.015	.021*	.016
% brain wt.	1.44	1.59	1.81	2.36*	1.46	1.76	1.82	1.83	2.69*	1.98
Brain (g)	86.5	81.7	78.9	69.8*	81.7	81.8	76.9	76.1*	70.9**	80.8
% body wt.	0.61	0.67	0.65	0.65	0.76	0.69	0.80	0.85	0.79	0.79
Heart (g)	101	93	98	79*	90	83	75	83	73	69
% body wt.	0.70	0.76	0.80	0.73	0.82	0.69	0.78	0.90*	0.80	0.67
% brain wt.	119	114	125	113	110	101	98	109	103	85
Kidneys (g)	64.8	52.8	63.3	59.6	59.1	44.7	43.1	45.7	48.5	42.0
% body wt.	0.44	0.43	0.51	0.55	0.53	0.34	0.45*	0.49**	0.53**	0.41
% brain wt.	76	65	81	85	72	54	56	61	68	52
Liver (g)	316	284	302	357	283	261	222	261	305	274
% body wt.	2.2	2.3	2.5	3.3**	2.6	2.2	2.3	2.8*	3.3**	2.7*
% brain wt.	369	349	389	510*	346	318	289	347	430	341

† = 4 dogs/sex/group

Statistical Significance: $p<0.05$ = *; <0.01 = **

Data extracted from Report Tables 18 and 19, pages 118 and 119.

15 ~~43~~

At 50 mg/kg/day, male and female absolute brain weights were below ($p<0.05$ or 0.01) respective control values. As relative weights were similar to controls, the difference in absolute weights is considered to be due to a lower final body weight at this dose level.

Significantly ($p<0.05$) lower heart absolute weight in males and higher relative (to body) weights in females at 50 mg/kg/day, do not appear to present a pattern which would indicate an effect of test article administration.

Males did not show much difference or patterns regarding absolute or relative kidney weights. Female relative-to-body weights were increased ($p<0.05$ or 0.01) over the control value at 10, 50 and 100 mg/kg/day. There was a suggestive increase in the absolute and relative-to-brain weights at 50 mg/ kg/day.

Liver weights in males (absolute not significant, relative-to-body weight <0.01 and relative-to-brain weight <0.05) at 50 mg/kg/day were greater than the control group values. In females, the 50 mg/kg/day liver weights were also greater than controls (absolute not significant, relative-to-body weight <0.01 and relative-to-brain weight not significant). In addition, relative-to-body weight livers, at 10 and 100 mg/kg/day were greater ($p<0.05$) than controls.

3. Microscopic

Table 9 presents those microscopic findings that were considered to be possibly related to test article administration.

As noted in Table 9 in dogs treated with R-4572, the brain and spinal cord were found to have microscopic findings seldom noted in controls. The animals involved were primarily in the 50 and 100 mg/kg/day gr ups. Eosinophilic bodies and vacuolation in the medulla were the most prevalant brain findings with demyelination of various levels of the spinal cord being the most noted observation.

The epididymides had an increase in the number of dogs in the 10 and 50 mg/kg/day group (2 and 3, respectively) exhibiting epithelial cystic degeneration compared with one in each of the control and other dosed groups. Tubular atrophy of the testes was not observed in control dogs, but was reported in 2, 0, 1 and 2 dogs of the 1, 10, 50 and 100 mg/ kg/day groups, respectively.

In the kidneys, cortical tubular lipofuscin pigment was present in 2 or 3 dogs in all treated female groups compared with none in the control group. In males, this finding was seen in all 4 controls and 2 or 3 animals in all treated groups. Cortical chronic inflammatory cell infiltration was

008452

Table 9

MICROSCOPIC PATHOLOGY FINDINGS POSSIBLY RELATED TO THE ADMINISTRATION OF R-4572 TO DOGS FOR ONE YEAR

	Males					Females				
mg/kg/day =	0	1	10	50	100	0	1	10	50	100
BRAIN - Eosinophilic bodies in medulla	0	0	0	2	1	0	0	0	3	2
Vacuolation in medulla	0	0	1	2	1	1	0	0	2	1
Eosinophilic bodies in pons	0	0	0	1	0	0	0	0	1	0
Vacuolation in pons	0	0	0	1	0	0	0	0	0	0
EPIDIDYMIS - Epithelial cystic degeneration	1	1	2	3	1	-	-	-	-	-
KIDNEY - Cortical tubular lipofuscin pigment	4	3	2	2	2	0	2	2	3	2
Cortical chronic inflammatory cell infiltration	1	0	0	1	0	0	1	1	4	1
NERVE, SCIATIC - Demyelination	0	3	3	4	2	2	1	0	2	4
SPINAL CORD, CERVICAL - Demyelination	1	3	3	4	3	1	1	0	4	3
SPINAL CORD, LUMBAR - Demyelination	0	1	2	4	2	0	1	2	4	1
SPINAL CORD, SACRAL - Demyelination	0	0	0	3	0	0	0	1	3	0
SPINAL CORD, THORACIC - Demyelination	0	2	3	4	3	0	3	1	4	3
SPLEEN - Hemosiderosis	3	4	4	4	2	1	4	4	4	4
TESTES - Tubular atrophy	0	2	0	1	2	-	-	-	-	-

NOTE: 4 dogs/sex/group examined

Data extracted from Report Table 21, pages 123-137.

noted in 1, 1, 1, 4 and 1 female dogs at 0, 1, 10, 50 and 100 mg/kg/day, respectively; whereas, in males, one control and one at 50 mg/kg/day had the finding.

Hemosiderosis in the spleen was reported in one female control dog and in all 4 dogs of each of the female treated groups. In males, this finding was noted in 3, 4, 4, 4 and 2 dogs at 0, 1, 10, 50 and 100 mg/kg/day, respectively.

All other microscopic findings were considered to be within normal limits or not the results of test article administration.

The Reviewer has the following comment regarding the Materials, Methods and Results:

This study is to be considered CORE SUPPLEMENTARY because there are no analyses data accompanying the report which show test article purity or stability. If these data are provided by the Registrant and are acceptable to the Agency, the study may be upgraded to CORE [illegible]M.

A brief description of s atistical analyses used in the study was provided.

A Good Laboratory Practice Compliance Statement, a Quality Assurance Statement and a list of Quality Assurance inspections were included in the report.

II. Discussion

The Registrant needs to provide the Agency with analyses data regarding test article purity and stability.

All dogs survived the one-year study. Animals dosed with 100 mg/kg/day (highest dose tested) had severe toxic effects and test article administration was stopped at 14 weeks with these dogs receiving empty gelatin capsules for the remainder of the one-year study ("recovery" dogs).

Body weight gains were decreased by 100 mg/kg/day during the dosing period, but recovery was made during the approximate last 9 months of the study. At 50 mg/kg/day, a decrease in body weight gain occurred and tended to become more severe as the study progressed. No statistically significant decreases in body weights compared with control values were observed in the 1 or 10 mg/kg/day groups. However, there did appear to be a decrease in body weight gain at these two doses, with a review of individual dog weights at each interval indicating some heavier animals in the treated groups.

The nervous systems were affected by the 50 and 100 mg/kg/day regimens as evidenced by the following: ataxia, reduced locomotor activity, splayed hind limbs, tremors, eosinophilic bodies/vacuolation of the medulla/pons and spinal cord demyelination (at all cord levels examined).

An effect on sperm/testes was indicated by a decrease in ejaculate volume, suggested at 6 months at 50 and 100 mg/kg/day, and statistically ($p<0.05$) reduced at 12 months by 50 mg/kg/day. In addition, 50 mg/kg/day had a statistically ($p<0.05$) reduced percent of motile sperm at 12 months. The report stated that R-4572 was known to have produced a rodent specific effect on sperm. Testicular tubular atrophy was noted in 0, 2, 0, 1 and 2 dogs at 0, 1, 10, 50 and 100 mg/kg/day, respectively, suggesting the possibility of a test article effect.

Anemia was observed in dogs administered 50 and 100 mg/kg/day, at primarily the 3 month interval, with a return toward control values at 12 months. Histopathological changes in liver and spleen accompanied by an increase in erythrocyte osmotic fragility plus the reduction in RBCs, hemoglobin and hematocrits suggested the possibility of a mild hemolytic anemia.

Organ weight differences (treated from control) at 50 and 100 mg/kg/day, appear to be primarily the result of a decrease in body weight gain (lower terminal body weight).

III. Conclusions

R-4572, administered orally by gelatin capsule at doses of 0, 1, 10, 50 and 100 mg/kg/day daily for one year to beagle dogs, caused the following adverse effects at 50 mg/kg/day and 100 mg/kg/day (dosed only for 14 weeks, then empty capsules): various effects on the nervous systems, a decrease in body weight gain, anemia and a decrease in ejaculate volume as well as the percent of motile sperm.

The Maximum Tolerated Dose (MTD) was considered to have been achieved: 50 mg/kg/day

The No Observed Effect Level (NOEL) = 10 mg/kg/day

The Lowest Observed Effect Level (LOEL) = 50 mg/kg/day
(statistically significant decreased body weight gain, adverse effects on the nervous systems, anemia and decreased ejaculate plus percent of mobile sperm)

Classification: CORE SUPPLEMENTARY - This may be upgraded if the Registrant provides acceptable data regarding test article purity and stability.

This study does not satisfy the Guideline Requirements (§83-1) for a chronic oral toxicity study in dogs.