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DATA EVALUATION REPORT

STUDY TYPE: 83-1(b) One Year Dog Study TOX. CHEM NO: 160A

ACCESSION NUMBER: None MRID NO.: 00129507

TEST MATERIAL: Carbofuran Technical, 96.1% purity

SYNONYMS: Furadan Insecticde, FMC 10242

STUDY NUMBER: Toxicogenics Study No. 410-0715

SPONSOR: FMC Corporation

TESTING FACILITY: Toxigenics, Inc., Decatur, IL

TITLE OF REPORT: One-Year Chronic Oral Toxicity Study in Beagle Dogs with Carbofuran

AUTHOR(S): Gale D. Taylor

REPORT ISSUED: June 6, 1983

CONCLUSION: Randomized groups of 6/sex/dose purebred beagle dogs, 6-8 months of age, received by diet daily doses of 0 (Purina Certified Canine Diet #5007), 10, 20, or 500 ppm of technical carbofuran for one year (0.25, 0.50, and 12.5 mg/kg/day). All animals were examined daily for mortality and clinical signs. Body weight and food consumption were measured weekly. Ophthalmological evaluations were performed every six months. Blood samples were taken monthly for hematology, clinical chemistries, and cholinesterase determinations. Urinalysis measurements were conducted every two months. All animals were sacrificed, grossly necropsied and tissues examined microscopically.

The cholinesterase LOEL is 10 ppm based on inhibition of plasma ChE in males. At 500 ppm, plasma, RBC (males), and brain (males) cholinesterase were significantly decreased. **The systemic NOEL is 20 ppm and the LEL is 500 ppm.** The effects consisted of toxic signs, decreased body weight and food consumption in both sexes (high-dose dogs were fed control diet to sustain life), anemia in males (decreased hematocrit, hemoglobin, and RBC's), decreased total protein, calcium, sodium, and elevated potassium in males, decreased absolute brain and heart weight in males, alopecia in 1/6 of both sexes, decreased body fat in 2/5 males, testicular degeneration with aspermia and giant cell formation in 4/5 males, and lung inflammation in 5/5 males and 2/6 females. Consultation

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with the HED pathologist regarding the significance of the testicular lesions in the 500 ppm dogs indicates that the testicular degeneration can be attributed to the poor nutritional status of the high-dose dogs rather than to a direct toxic effect of carbofuran in these animals.

Classification: Core-Minimum

Special Review Criteria (40 CFR 154.7)

A. MATERIALS:

1. Test compound: Description - Carbofuran, Technical Grade, Batch # - M607210, Purity - 96.1%, Description - Light Brown, Crystalline Solid.

2. Test animals: Species: Dog, Strain: Purebred Beagle, Age: 6-8 Months, Weight: Five to Ten Kilograms, Source: White Eagle Laboratories, Doylestown, PA.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose in diet (ppm)	Main Study 12 months male	female	Interim Sac. months male	female
1 Control	0	6	6		
2 Low (LDT)	10	6	6		
3 Mid (MDT)	20	6	6		
4 High (HDT)	500	6	6		

2. Diet preparation

Diet was prepared weekly and stored at room temperature. Samples of treated food were analyzed for stability and concentration at monthly intervals.

Results - Analyses of sample test diets confirmed that diets were mixed homogeneously and that they were stable for 7 days. Over the one year chronic study period, among the monthly dietary analyses reported, only one diet exceeded the ± 20% range of the nominal concentration (treatment month, 10; nominal concentration, 10 ppm; assayed concentration, 12.3 ppm).

3. Animals received a pulverized basal diet (Purina Certified Canine Diet #5007) on a 2-hour per day feeding interval and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: Quantitative continuous variables were analyzed using Analysis of Variance (ANOVA). Significant differences determined by ANOVA were further

examined using Scheffe's (unequal population) or Tukey's (equal population) procedures. Non-continuous variables were analyzed by the Kruskal-Wallis Statistic Test. Significant differences determined by this test were further analyzed by the Kruskal-Wallis Multiple Comparison Test.

5. A quality assurance statement was signed by Jane D. McCarty and Walter L. Bullock on June 6, 1983.

C. METHODS AND RESULTS:

1. Observations:

Animals were inspected daily for signs of toxicity and mortality.

Toxicity/Mortality (survival)

1. Survival - One death occurred during the study. One male (Dog #AB5324) died following 201 days of exposure to the 500 ppm carbofuran test diet. A pronounced deterioration in the health of this animal was observed in the week before death. At necropsy, the body was emaciated and dehydrated. Microscopic pathology revealed atrophy, hypoplasia, and discoloration of numerous tissues and organs plus a mild renal mineralization and adrenal cortical degeneration. Death was considered directly due to ingestion of carbofuran. In order to protect against further reductions in body weight and to prevent additional deaths, the remaining animals in the 500 ppm groups were provided supplemental control diet throughout the study until termination. The frequency and consumption of control diet provided the 500 ppm animals were not reported.

2. Toxicity

	Males				Females			
Dose (ppm)	0	10	20	500	0	10	20	500
Observations -Total								
Emesis	0	0	2	68	0	0	0	56
Emaciation	0	0	0	35	0	0	0	11
Loose stool	0	0	2	32	0	0	0	19
Muscle tremors	0	0	0	7	0	0	0	4
Salivation	0	0	0	6	0	0	0	7

Toxicity was essentially limited to high-dose males and females, as can be seen in the above Table. The NOEL for toxic signs is 20 ppm in both sexes.

2. Body weight

Animals were weighed weekly for the duration of the study. Results: Significantly lower body weights were reported for the 500 ppm males from week 20 to termination. At the end of the study, body weight losses for the high-dose males and females were significant. The NOEL for body weight is the 20 ppm group for both sexes. Body weight data are summarized in the Table below:

Mean Body Weight (Kg)

Group	WEEKS						
Male (PPM)	1	10	20	30	40	Final	Change
0	7.5	8.8	9.3	9.6	9.6	10.0	2.5
10	7.8	9.1	9.6	10.0	9.9	10.2	2.4
20	7.7	9.0	9.3	9.6	9.6	9.9	2.2
500	7.5	7.2	6.1**	7.0[a]	6.5*	6.6	-0.9
Female (PPM)							
0	6.3	7.1	7.5	7.7	8.0	7.8	1.5
10	6.7	7.2	7.5	7.6	7.7	7.8	1.1
20	6.4	7.0	7.3	7.1	7.4	7.8	1.2
500	6.9	6.6	6.2	7.0	6.4	6.5	-0.4

* $p < 0.05$
** $p < 0.01$
a = one male died during week 29

3. Food consumption and compound intake

Food consumption was recorded weekly and carbofuran intake was calculated. The following Table of mean daily food consumption in grams shows that food consumption was decreased in both sexes at 500 ppm at week 44. The NOEL for food consumption is 20 ppm for both sexes.

Group	WEEKS			
Male (PPM)	1	13	30	44
0	241	224	224	177
10	256	229	227	180
20	209	219	225	189
500	182	210	217	113**
Female (PPM)				
0	278	254	238	186
10	238	214	184	182
20	249	218	222	219
500	204	255	212	109*

* $p < 0.05$
** $p < 0.01$

4. Ophthalmological examination

Performed at 6 month intervals on all animals.

Results: No apparent treatment-related findings were observed during these examinations.

5. Blood was collected before treatment and at monthly intervals for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology

X	
x	Hematocrit (HCT)*
x	Hemoglobin (HGB)*
x	Leukocyte count (WBC)*

X	
x	Leukocyte differential count*
	Mean corpuscular HGB (MCH)
	Mean corpusc. HGB conc. (MCHC)

x	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
x	Platelet count*	x	Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies

Results - Hematocrit, hemoglobin, and erythrocyte values were significantly decreased in the 500 ppm males, beginning with month 5 and continuing to the end of the study. Less significant reductions were seen in 500 ppm females at months 6 and 8. Reticulocytes were decreased in females at 4, 5, 8, and 11 months. Hematology values were normal at other times. The NOEL for hematological values is 20 ppm in both sexes. The following Table summarizes the results.

MALES

Parameter / PPM	1 Month 0	1 Month 500	6 Month 0	6 Month 500	12 Month 0	12 Month 500
Hematocrit (%)	41.0	41.2	44.6	37.6**	47.6	37.8**
Hemoglobin (G/dL)	14.8	14.8	16.6	11.8**	16.9	13.1**
RBC (10^6/cmm.)	6.59	6.47	7.16	5.25**	7.40	5.80**
Reticulocyte (%)	0.9	1.0	0.9	1.0	0.7	0.6

FEMALES

PPM	0	500	0	500	0	500
Hematocrit (%)	43.6	45.2	45.1	39.1*	48.1	43.0
Hemoglobin (G/dL)	15.7	16.5	16.9	14.6*	17.1	15.7
RBC (10^6/cmm.)	6.68	7.18	6.98	6.38	7.10	6.87
Reticulocytes (%)	1.2	0.9	1.1	0.8	0.8	0.5

* $p < 0.05$
** $p < 0.01$

b. Clinical Chemistry

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

	Enzymes
x	Alkaline phosphatase (ALK)
x	Cholinesterase (ChE)#
	Creatinine phosphokinase*^
x	Lactic acid dehydrogenase (LAD)
x	Serum alanine aminotransferase (also SGPT)*
x	Serum aspartate aminotransferase (also SGOT)*
	Gamma glutamyl transferase (GGT)
	Glutamate dehydrogenase

X	Other:
x	Albumin*
	Blood creatinine*
x	Blood urea nitrogen*
x	Cholesterol*
x	Globulins
x	Glucose*
x	Total bilirubin
x	Total serum Protein (TP)*
	Triglycerides
	Serum protein electrophores

* Required for subchronic and chronic studies
# Should be required for OP: Plasma, RBC, and Brain
^ Not required for subchronic studies

Results - Cholinesterase

1. Plasma Cholinesterase: A significant depression of plasma cholinesterase was observed in the 10 ppm males in comparison to controls at the following intervals:

Study Day	Percent Reduction
3	18
7	20
14	19

Study Month	Percent Reduction
1	19
2	17
3	19
4	18
5	17

Plasma cholinesterase was comparable to controls in 10 ppm males at other times. Plasma cholinesterase was not significantly depressed in females at 10 ppm in comparison to controls at any sampling interval.

At 20 ppm, in females, at only month 8, there was a 26 % decrease in plasma cholinesterase. In males at 20 ppm, plasma cholinesterase was significantly decreased at most sampling intervals, as shown below:

Study Day	Percent Reduction
3	25
7	27
14	21

Study Month	Percent Reduction
1	31
2	25
4	22
5	29
6	24
7	25
8	26
9	23
10	24
11	30
12	30

At 500 ppm, plasma cholinesterase was decreased in both sexes at all times by values equal to or greater than 77%. The decreases in plasma cholinesterase at 20 and 500 ppm are considered compound-related. The LOEL for plasma cholinesterase in males is 10 ppm.

2. RBC Cholinesterase: At 500 ppm, males had significant decreases in RBC cholinesterase at month 5 (21%), month 6 (26%), and month 11 (24%). These decreases in RBC cholinesterase at 500 ppm are considered treatment-related.

3. Brain Cholinesterase: A 24% decrease in males and a 44% increase in females was observed in brain cholinesterase at termination. The toxicological significance of the findings in females is not known. However, it should be recalled that high-dose animals were fed control diets to sustain life as a supplement to the 500 ppm carbofuran diets. In females, the increase in brain cholinesterase may reflect a "rebound" effect from brain cholinesterase depression due to the ingestion of control diet. However, the decrease in brain cholinesterase in male dogs is considered treatment-related.

4. Other Clinical Chemistry Results: Other consistent findings in males at 500 ppm were decreases in total protein at 5, 8, and 12 months (females, also, at this period); decreases in calcium at 3, 5, 7, 8, 9, 10, 11, and 12 months; decreases in sodium at 2, 4, 6, 7, 9, 10, and 12 months; and increases in potassium at 6 and 10 months. Other inconsistent significant findings in clinical chemistries were not considered treatment-related. The NOEL for clinical chemistries, other than cholinesterase, is 20 ppm.

6. Urinalysis^

Urine was collected from fasted animals at 60 day intervals. The CHECKED (X) parameters were examined.

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

^Not required for subchronic studies
* Required for chronic studies

Results - Most urinalyses results were within the normal range for dogs and isolated individual findings were not consistently observed in a dose-related fashion. The NOEL for urinalysis is 500 ppm.

7. Sacrifice and Pathology

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
	Tongue	x	Aorta*	xx	Brain*+
x	Salivary glands*	xx	Heart*	x	Periph. nerve*#
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*#
x	Stomach*	x	Lymph nodes*	xx	Pituitary*
x	Duodenum*	x	Spleen	x	Eyes (optic n.)*#
x	Jejunum*	x	Thymus*	Glandular	
x	Ileum*	Urogenital		xx	Adrenal gland*
x	Cecum*	xx	Kidneys*+		Lacrimal gland#
x	Colon*	x	Urinary bladder*	x	Mammary gland*#
	Rectum*	xx	Testes*+	xx	Parathyroids*++
xx	Liver *+	x	Epididymides	xx	Thyroids*++
x	Gall bladder*	x	Prostate	Other	
x	Pancreas*		Seminal vesicle	x	Bone*#
Respiratory		xx	Ovaries*+	x	Skeletal muscle*#
x	Trachea*	x	Uterus*	x	Skin*#
x	Lung*			x	All gross lesions and masses*
	Nose^				
	Pharynx^				
	Larynx^				

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement.
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

a. Organ weight - In male dogs, the absolute brain weight was decreased by 14%, and the absolute heart weight was decreased by 38%, which were significant. However, the relative organ to brain and organ to body weight ratios were not significantly different. There were no other significant effects in organ weights in either sex of dog. Also, there was no histopathology associated with the decreased organ weights at 500 ppm. The NOEL is 20 ppm for organ weight changes.

b. Gross pathology - Necropsy showed 2/5 males with lack of body fat and alopecia in 1/5 males and 1/6 females at 500 ppm. These findings are considered treatment-related.

The NOEL for necropsy findings is 20 ppm.

c. Microscopic pathology - There were no neoplastic findings, and the non-neoplastic findings associated, definitively, with treatment were testicular degeneration and lung inflammation at 500 ppm. The hepatocellular cytoplasmic atypia with prominent centrilobular pattern of distribution, suggestive of increased amounts of endoplasmic reticulum, was observed in 2/12 controls, 9/10 10 ppm dogs, 7/12 20 ppm dogs, and 6/11 500 ppm dogs. This finding was not considered toxicologically significant due to the absence of a dose-related response with grade, the absence of elevated liver weight and liver enzymes, and the fact that there was no indication of progression to degeneration or necrosis.

1) Non-neoplastic - Testes, Lung, and Liver

1. Testes

	Dose (ppm)			
	0	10	20	500
Dog No.	-	AB5330	AB5365	AB5317 AB5329 AB5332 AB5697
Findings	-	Aspermia, epidid./U.L., focal,severe	Aspermia, degeneration, sem. tub., diffuse, mild	Aspermia, Giant cell formation, degener., moderate
Incidence	0/6	1/6	1/6	4/5

Testicular degeneration, aspermia, and giant cell formation were considered compound-related in the 4/5 males at 500 ppm. However, the incidence of 1/6 at both the 10 and 20 ppm dose levels were not significantly different ($p > 0.05$) from the control incidence of 0/6. Therefore, the NOEL for testicular effects is 20 ppm.
Consultation with the HED pathologist regarding the significance of the testicular lesions in the 500 ppm dogs indicates that the testicular degeneration can be attributed to the poor nutritional status of the high-dose dogs rather than to a direct toxic effect of carbofuran in these animals.

2. Lung - Inflammation

Dose (ppm)	0		10		20		500	
	m	f	m	f	m	f	m	f
Incidence	0/6	0/6	1/6	2/6	0/6	1/6	5/5	2/6
Grade of Lesion			min.	mild mild		min.	mild min. mild mod. min.	mod. mod.

At 500 ppm, the inflammation in the lungs occurred in 5/5 males and 2/6 females and there was an increase in the grade of the lesion over the other treated groups. The NOEL for lung inflammation is considered to be 20 ppm.

3. Liver - Cytoplasmic atypia with centrilobular pattern

Dose (ppm)	0		10		20		500	
	m	f	m	f	m	f	m	f
Grade of Lesion								
mild	0	0	2	1	0	2	0	1
moderate	0	0	1	3	1	2	0	2
severe	0	0	2	0	1	1	0	2
very severe	0	2	0	0	0	0	1	0
Incidence	0/6	2/6	5/6	4/6	2/6	5/6	1/5	5/6

In males, the distribution of the lesion is not dose-related, although there are no lesions in the control. In females, the most severe lesion occurred in the controls (2/6 were very severe). Also, in females, the severity of the grades of the lesion in the treated dogs do correspond to dose, although the incidence of inflammation in females are comparable in treated groups. Additionally, there was no increase in liver enzymes or any changes in liver weight to correspond to the cytoplasmic atypia in the liver. The atypia did not progress to cytoplasmic degeneration or necrosis. For these reasons, the findings in the liver are not considered toxicologically significant. The NOEL is 500 ppm.

2) Neoplastic - There were no neoplasms in the study.

D. DISCUSSION: The study was well conducted, although the selection of the dose levels can be criticized, and is acceptable. Individual animal data were available and there was a thorough statistical analysis by the testing facility of all measured criteria. This study has previously been used as the basis of the RfD. The cholinesterase LOEL is 10 ppm based on plasma cholinesterase depression in males. At 500 ppm, plasma, RBC (males), and brain (males) cholinesterase were significantly decreased. The systemic NOEL is 20 ppm and the LEL is 500 ppm. The effects consisted of toxic signs, decreased body weight and food consumption in both sexes (high-dose dogs were fed control diet to sustain life), anemia in males (decreased hematocrit, hemoglobin, and RBC's), decreased total protein, calcium, sodium, and elevated potassium in males, decreased absolute brain and heart weight in males, alopecia in 1/6 of both sexes, decreased body fat in 2/5 males, testicular degeneration with aspermia and giant cell formation in 4/5 males, and lung inflammation in 5/5 males and 2/6 females.