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CONFIDENTIAL BUSINESS INFORMATION
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MRID: 00001421

DATA EVALUATION RECORD

(1) CHEMICAL: Chloroneb 09/24

(2) TYPE OF FORMULATION: Commercial--Fungicide 1823, a 65 or 75% wettable powder

(3) CITATION: Busey, W.M., and Kundzins, W. 1967. Two-Year Dietary Feeding--Dogs: Fungicide 1823: Final Report: Project No. 201-125. (Unpublished study received July 8, 1968, under 8F0657; prepared by Hazleton Laboratories, Inc., submitted by E.I. du Pont de Nemours and Co., Wilmington, Del.; CDL:091147-A)

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(6) TOPIC: This study has information pertinent to discipline toxicology, topic subchronic oral toxicity. It relates to the Proposed Guidelines data requirement 163.82-1.

(7) CONCLUSION: Fungicide 1823 was fed to male and female beagle dogs at dietary levels of 100, 500, and 2,500 to 10,000 ppm active ingredient for 2 years. The appearance, behavior, appetite, elimination, body weight changes, clinical laboratory studies, organ weights, organ/body weight ratios, gross necropsy, and histopathologic findings were observed or calculated and recorded.

No signs of toxic effects from the consumption of Fungicide 1823 at the low (100 ppm) or intermediate (500 ppm) levels were observed. Loss of body weight occurred in the high dietary level group when fed 7,500, 8,750, and 10,000 ppm concentrations. In three of six high level dogs at 18 months, serum glutamic-pyruvic transaminase and/or alkaline phosphatase activities were moderately to markedly elevated. At necropsy the mean liver weights and ratios of the high level dogs were moderately elevated when they were compared with the controls. Mean adrenal weights and ratios of the high level dogs were slightly elevated.

Histopathologic changes associated with the feeding of Fungicide 1823 at the high dose level (2,500 to 10,000 ppm) were observed in the liver, thryoid, and stomach of

some dogs killed at 2 years. Morphologic changes in the thyroid, indicative of a moderate increase in activity, were characterized by uniform, small-to-medium-sized follicles, which were lined by medium-to-high cuboidal epithelium in 4 of 6 dogs. The livers from 4 of 6 dogs had moderate-to-severe pigmentation consisting of small yellowish brown granules in hepatocytes. A moderate subacute-to-chronic catarrhal gastritis was observed in stomachs from 4 of 6 dogs.

In this study, the no-observed-effect level was 500 ppm (12.5 mg/kg body weight per day). The study is judged an adequate subchronic test in dogs.

CORE CLASSIFICATION: Minimum Data. This study used too few animals to be classified Guideline.

(8) MATERIALS AND METHODS:

(a) Test Materials: Fungicide 1823 was supplied by E.I. du Pont de Nemours Co. Three batches were used, Fungicide 1823 (75% WP), Fungicide 1823 (65% WP) Sample A, and (65% WP) Sample C. Fungicide 1823 (75% WP) was fed from the beginning of the study to week 30; Sample A was fed from week 30 to week 99; Sample C was fed from week 99 to the end of the study.

(b) Animals: Thirty-two adult purebred beagle dogs, 16 males and 16 females, having initial weights of 5.8 to 11.5 kg were used.

(c) Control Group: The control group of dogs was untreated and fed the basal diet of ground Wayne Dog Meal alone.

(d) Duration of the Test: The study was 24 months with an interim sacrifice of selected dogs at 12 months.

(e) Number of Dose Levels:

Group No.	Number of Dogs		Dietary Level (ppm active ingredient)	Weeks Fed
	Male	Female		
1 (Control)	4	4	0	1-104
2	4	4	100	1-104
3	4	4	500	1-104
4	4	4	2,500	1-32
			3,500	33-34
			5,000	35-58
			7,500	59-68
			8,750	69-70
			10,000	71-85
			7,500	86-104

(f) Route of Administration: Fungicide 1823 was incorporated in the basal diet of ground Wayne Dog Meal on a weight/weight basis and thoroughly mixed in a twinshell blender to provide the appropriate dietary level. Fresh mixtures were prepared weekly. The diet and water were freely available at all times.

(g) Caging: The dogs were housed individually in metal cages.

(h) Observations: The appearance, behavior, appetite, and elimination were observed daily. Both food and compound consumption and body weight were recorded weekly.

(i) Clinical Laboratory Studies: Blood and urine specimens were collected for evaluation three times initially, 1 week apart, and at 1, 2, 3, 6, 12, 18, and 24 months on all dogs in each group.

(j) Hematology: Hematocrit, hemoglobin, erythrocyte counts, and total and differential leukocyte counts were determined.

(k) Biochemistry: Bromsulphalein liver function, blood sugar, blood urea nitrogen, serum alkaline phosphatase, serum glutamic-pyruvic transaminase, prothrombin time, total protein, albumin, globulin, and albumin/globulin ratio values were determined.

In addition, protein-bound iodine was determined for the control and high level (2,500 to 10,000 ppm) dogs at 24 months only.

(l) Urinalysis: Appearance, pH, specific gravity, sugar, acetone, protein, bilirubin, occult blood, and microscopic appearance of the sediment were evaluated.

(m) Interim Sacrifice: Eight dogs (one male and one female from each group) were sacrificed at 1 year. Necropsies were performed, and tissues were collected in the same manner as at terminal sacrifice.

(n) Terminal Sacrifice: The remaining 24 dogs (3 males and 3 females from each group) were sacrificed at 2 years and necropsied grossly.

(o) Organ Weights: Brain, pituitary, thyroids, thymus, lung, heart, liver, spleen, kidneys, adrenals, testes, and ovaries were weighed and organ/body weight ratios were calculated for each dog.

(p) Residue Analysis: Brain, liver, spleen, kidneys, testes, fat, muscle, blood, feces, and urine were saved for chemical analysis.

(q) Tissues Preserved: In 10% buffered neutral formalin the brain, pituitary, spinal cord, eyes, salivary gland, thyroids, parathyroids, thymus, lung, heart, liver, gall-bladder, spleen, kidneys, adrenals, stomach, pancreas, duodenum, jejunum, ileum, colon, mesenteric lymph node, urinary bladder, prostate (at terminal sacrifice only), ovaries, uterus, bone (costochondral junction) bone marrow (sternum), sciatic nerve, and surrounding muscle were fixed.

The testes were fixed in Bouin's solution,

(r) Histopathologic Examination: All cited tissues except the eyes were examined from the control and high dietary level dogs. Target organs only (thyroid, liver, stomach, and small intestine) were examined from dogs in the low (100 ppm) and intermediate (500 ppm) groups.

(9) REPORTED RESULTS:

(a) Appearance and Behavior: No signs of toxic effects from the consumption of Fungicide 1823 were observed in the low or intermediate group dogs. Dogs on the high dietary level lost body weight during the period of weeks 59 through 85 when the concentrations fed were raised from 5,000 to 7,500, 8,750, and 10,000 ppm. When the level was decreased from 10,000 to 7,500 ppm (weeks 86 through 104), the dogs recovered some of the lost weight.

(b) Food consumption for the high level dogs was generally comparable with control measurements.

The following table gives the levels of daily intake of fungicide. All levels were calculated from measured food consumption.

Table 1. Daily Intake of Fungicide 1823

Male

Weeks of Test	No. of Dogs	Dietary Level (ppm) 100	500	2,500-10,000
1-32	4	1.8-5.1[a,b]	7.7-25	42-98[c]
33-52	4	1.7-5.5	6.7-20	86-190[d,e]
53-71	3	1.9-4.8	11-22	120-250[e,f]
72-84	3	1.3-4.6	11-19	160-570[g,h]
85-104	3	0.8-4.5	6.9-20	210-530[i]
1-32	4	1.5-4.3	6.6-20	40-110
33-52	4	1.9-4.8	9.1-19	47-220
53-71	3	2.0-3.5	10-18	110-230
72-84	3	1.9-3.9	8.9-21	170-410
85-104	3	1.5-3.5	5.7-26	130-280

[a]Mg/kg body weight of Fungicide 1823 consumed daily

[b]Highest and lowest value during the time period given

[c]Dietary level 2,500 ppm, weeks 1 through 32

[d]Dietary level 3,500 ppm, weeks 33-34

[e]Dietary level 5,000 ppm weeks 35-58

[f]Dietary level 7,500 ppm, weeks 59-68

[g]Dietary level 8,750 ppm, weeks 69-70

[h]Dietary level 10,000 ppm, weeks 71-85

[i]Dietary level returned to 7,500 ppm, weeks 86-104

(c) Hematology: The values for test and control dogs generally remained within accepted limits of normal variance and were comparable among the group. One male high level dog had a low hemogram at termination, which was believed to be a reflection of his poor physical state. This dog had lost 3.3 kg during the 2-year test.

(d) Biochemistry: Bromsulphthalein liver function tests, blood sugar, blood urea nitrogen, prothrombin time, total protein, albumin, globulin, and albumin/globulin ratios were within acceptable limits of normal for all groups. Moderately to markedy elevated levels for serum glutamic-pyruvic transaminase and/or alkaline phosphatase were noted in three high level dogs (one male and two females) at 18 months. One low level female had a slightly elevated serum glutamic-pyruvic transaminase value at 12 months.

Serum transminase and phosphatase activities were within normal limits for the control and other treated dogs. No apparent trend was evident in the results of the protein-bound iodine determinations.

(e) Urinalyses: The values for test and control dogs generally were within normal limits and were comparable for all groups.

(f) Group Necropsy Findings: Gross examinations at necropsy at the 1-year interim and 2-year terminal sacrifices revealed only tissue alterations among test dogs that were judged by the examiner to be incidental and not compound related.

(g) Organ Weights: The mean liver weights and ratios of the high level (2,500-10,000 ppm) dogs were moderately elevated when they were compared with the controls. Mean adrenal weights and ratios of the high level dogs were slightly elevated when compared with control measurements. The other values were within limits of normal variation for control and treated dogs.

(h) Histopathology: Compound-related histopathologic changes were found in the liver, thyroid, and stomach of some of the dogs in the high dosage group killed after 2 years on test diet. In the thyroid, evidence of increased activity was observed in 4 of 6 dogs. These thyroids had rather uniform, small-to-medium-sized follicles that were lined by medium-to-high cuboidal epithelium. This was in contrast to the thyroids of the control and other test dogs that had variable-sized follicles lined, for the most part, with low-cuboidal epithelium. The thyroid of one other male dog at 100 ppm also showed slight increased activity that was not considered compound related. Two dogs, one male control and one female high dose group, had a moderate thyroiditis which was considered spontaneous in origin.

Moderate-to-severe pigmentation was seen in the livers of two of three males and two of three females in the high dose group. This pigmentation consisted of small yellowish brown granules in the cytoplasm of some of the

hepatocytes. The livers of the other male and female in the high dose group had slight pigmentation. Similar pigments were present in minimal to slight amounts in five of six dogs in the control group and in all 12 dogs in the low (100 ppm) and intermediate (500 ppm) level groups.

Moderate catarrhal gastritis was present in the stomach of two of three males and two of three females in the high dose group. These were subacute-to-chronic gastritis characterized by a moderately increased mucus production accompanied by a minimal mononuclear inflammatory cell infiltrate in the lamina propria. The stomach of the other female in the high dose group had a similar but milder catarrhal gastritis. Slight-to-moderate chronic gastritis characterized by a focal increased lymphocytic infiltrate was present in two of six controls, two of six low dose dogs, one of six intermediate dogs, and two of six high dose dogs. The changes were considered spontaneous.

No changes attributable to Fungicide 1823 were noted in the thyroid, liver, and stomach from the dogs in the low (100 ppm) and intermediate (500 ppm) groups.

Other histopathologic changes occurred in all groups including the control dogs and were considered spontaneous with no relationship to the feeding of Fungicide 1823.

(10) DISCUSSION: The purpose of this study was to characterize and evaluate long-term oral toxicity of Fungicide 1823 in dogs. This purpose was accomplished. It adequately assessed the potential hazard of Fungicide 1823 in the dog for present Guideline 163.82-1 requirements. The experimental design is good and the parameters evaluated were those generally evaluated by industrial toxicologists at the time of this report.

There are some weaknesses in the study that reduce the effectiveness of the report. For example, the purity of the test material was not confirmed and the amount of active ingredient was assumed to be the same as that listed on the label. The nature of contaminants and other materials is unknown. The manufacturing grade of the chemical was not given. Nor were the diets analyzed for content of test chemical or for stability of test chemical in diet.

(11) TECHNICAL REVIEW TIME: 6.0 hours