12-11-91

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

008894

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: BROMACIL (DPX-N 976-136) - A One-Year Feeding Study in Dogs (Guideline §83-1)

Caswell No.: 111 **MRID No.:** 418697-01
HED Project No.: 1-1384 **Identification No.:** 012301

FROM: Alan C. Levy, Ph.D., Toxicologist Alan C. Levy
Review Section IV, Toxicology Branch II Dec. 6, 1991
Health Effects Division (H7509C)

TO: Lois Rossi/Mario Fiol, PM 70
Registration Division (H7508W)

THRU: Elizabeth A. Doyle, Ph.D., Section Head E.A. Doyle
Review Section IV, Toxicology Branch II 12/6/91
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief M van Gemert
Toxicology Branch II 12/11/91
Health Effects Divsion (H7509C)

REQUEST: Review a one-year feeding study in dogs with BROMACIL.

Registrant: E. I. du Pont de Nemours and Company

CONCLUSIONS:

BROMACIL, administered by dietary admix at concentrations of 0, 25, 150 and 625 ppm to dogs for one year, appeared to cause a slight decrease in body weight gains in males and especially in females at 625 ppm. In addition, there is the suggestion that ail

three concentrations may increase the incidence of testicular atrophy/degeneration.

The **No Observed Effect Level (NOEL)** = <25 ppm (0.625 mg/kg), lowest dose tested - suggestion of possible testicular atrophy/ degeneration.

The **Lowest Observed Effect Level (LOEL)** = 25 ppm, lowest dose tested - testicular atrophy and degeneration

Classification: **Core Supplementary.** This is due to testicular observations reported at all concentrations tested. The Registrant may wish to present to the Agency additional data to substantiate that this finding (at this incidence - 3/5 in each group versus 0/5 in control) is not likely to have been the result of BROMACIL administration.

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

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy Dec. 6, 1991
Section IV, Tox. Branch II (H7509C)

Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 12/6/91
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

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

TEST MATERIAL: BROMACIL TOX. CHEM. NO.: 111

SYNONYMS: IN N976-136, DPX-N976-136 HED PROJECT NO.: 1-1384
MRID NO.: 418697-01
IDENTIFICATION NO.: 012301

STUDY NUMBERS: Medical Research Project No.: 8676-001
Haskell Laboratory Report No.: 1-91

SPONSOR: Agricultural Products
E. I. du Pont de Nemours and Company

TESTING FACILITY: E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P.O. Box 50
Newark, DE 19714

TITLE OF REPORT: Chronic Toxicity Study with Bromacil (DPX-N 976-136) - One-Year Feeding Study in Dogs

AUTHOR: Matthew S. Bogdanffy

REPORT ISSUED: February 12, 1991

CONCLUSIONS:

Bromacil administered by dietary admix at concentrations of 0, 25, 150 and 625 ppm to dogs for one year appeared to cause a slight decrease in body weight gains in males and especially in females at 625 ppm. In addition, there is the suggestion that all three concentrations may increase the incidence of testicular atrophy/ degeneration.

The **No Observed Effect Level (NOEL)** = <25 ppm (0.625 mg/kg), lowest dose tested - suggestion of possible testicular atrophy/ degeneration.

The **Lowest Observed Effect Level (LOEL)** = 25 ppm, lowest dose tested - testicular atrophy and degeneration.

Classification: **Core Supplementary.** This is due to testicular observations reported at all concentrations tested. The Registrant may wish to present to the Agency additional data to substantiate that this finding (at this incidence - 3/5 in each group versus 0/5 in control) is not likely to have been the result of BROMACIL administration.

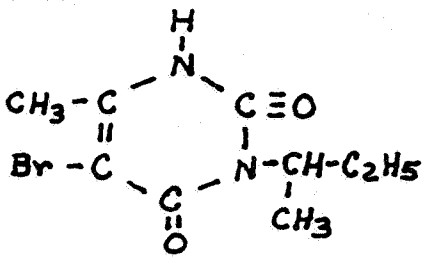
This study does not satisfy the Guideline Requirements (§83-1) for a chronic oral toxicity study in dogs.

I. MATERIALS, METHODS AND RESULTS

A. Test Article Description

Name: 2,4 (1H, 3H) - Pyrimidinedione, 5-bromo-6-methyl-3-(1-methylpropyl)- BROMACIL, IN N976-136, DPX-N976-136

Formula:



Lot Number: T07128852

Purity: 95.9%

B. Dose Selection

The concentrations used in this one-year dog study were 0 (control), 25, 150 and 625 ppm (weight/weight concentration of active ingredient BROMACIL adjusted for 95.9% purity).

In a previous dog study of 1-2 years duration (Medical Research Project No. 686), BROMACIL was administered as a dietary admix at concentrations up to 1,250 ppm. There was a decrease in food consumption (particularly during the first 10 weeks of dosing) and a decrease in body weight gains (9-19%) at 1,250 ppm during the first year. Body weights appeared to recover to control levels after about two years. There was the suggestion of an effect on palatability. There were no other signs of toxicity.

A palatability study (Medical Research Project No. 8676) was performed using 1,250 and 2,500 ppm. There was a decrease in food consumption at both concentrations. The dogs were then given a control diet plus capsules containing 50 mg/kg doses of BROMACIL. [50 mg/kg = approximately 2,000 ppm]. Emesis occurred following capsule administration. In a previous acute study, emesis was observed after capsules of 100 mg/kg. Dogs were fed 625 ppm to verify the observation made in the first group (only minimal effects were observed). The Report states that, "Therefore, 625 ppm was chosen as the highest level for testing that would not induce effects on palatability likely to compromise interpretation of results."

C. Test Article Analyses for Purity, Stability and Homogeneity

Representative data for these parameters are presented in Table 1.

Table 1

A SUMMARY OF ANALYTICAL DATA FOR PURITY, STABILITY AND HOMOGENEITY OF BROMACIL IN A ONE-YEAR DOG STUDY

	Nom.	det.	average	range	average	range
RECOVERY OF BROMACIL ADDED TO CONTROL	25	12	25.3	23.2-27.5	101.0	94-109
	625	12	617.8	560-648	98.8	91-103
CONCENTRATION IN FEEDER SAMPLES	25	10	23.7	21.1-26.8	94.2	82-107
	150	8	155.5	146-169	103.8	97-113
	625	8	605.6	577-628	96.5	92-100
	Nominal		Average of Two		Percent of Nominal	
STABILITY IN DIETS						
Fresh frozen	25		25.5		102	
7 day room temp.	25		28.4		114	
14 day refrig.	25		26.0		104	
Fresh frozen	625		598		96	
7 day room temp.	625		618		99	
14 day refrig.	625		607		97	
HOMOGENEITY						
Top	25	2,2	24.0,28.8		96,115	
Middle	25	2,2	23.8,23.4		95,94	
Bottom	25	2,2	26.6,24.2		106,97	
Top	150	2,2	133,148		89,99	
Middle	150	2,2	146,135		97,90	
Bottom	150	2,2	139,138		93,92	
Top	625	2,2	619,621		99,99	
Middle	625	2,2	630,585		101,94	
Bottom	625	4,2	833a,628		133,100	

a = Sample reanalyzed due to high recovery.
Nom. = Nominal
det. = No. of determinations
Data extracted from Report Analytical Tables I-V, page 38-43.

The purity, stability and homogeneity data presented in the report appear to indicate that the actual dietary concentrations were similar enough to the nominal concentrations to be acceptable.

D. Animals

Beagle dogs (22/sex), approximately 5 months of age, were received from Marshall Research Animals, North Rose, NY. At arrival, body weights were 7.1-9.2 kg for males and 6.2-7.7 kg for females. The animals were housed individually and fed Purina Certified Canine Diet #5007 plus tap water during a 77 day acclimation period. They were weighed 11 times during this period, observed daily, had ophthalmoscopic examinations (test day -74) and had clinical pathology evaluations on test days -75 and -60.

On test day -1, the dogs were placed 5/sex into four groups based on body weights, clinical pathology measurements, ophthalmological evaluation and a general physical examination. [Randomization - The report states, "Differences in these parameters between each group within a sex were minimized."] The average age of the dogs at the start of the study was 237 days (approximately 8 months).

During the study, each animal received about 350 g of diet/day. Daily exercise was provided in an outdoor run, weather permitting. Targeted room temperature was 23 ± 2° C with relative humidity of 50 ± 10% and a 12-hour light/dark cycle.

Diets were prepared weekly and refrigerated until used.

E. General Observations

1. Mortality and Clinical Signs - The dogs were observed at least once/day.

Dog No. 2905 (625 ppm male) was sacrificed <u>in extremis</u> on day 99. The animal had been treated on test days 87-99 with ampicillin for a condition thought to be an infection (body weight loss since day 56, anorectic and feverish). Later diagnosis was "spontaneous disseminated panarteritis."

Dog No. 2910 (control male) was treated for a leg wound (Epsom salts and hot water soak on day 259).

It is not considered that any of the above mentioned observations (or moribund sacrifice) or any other clinical signs noted were attributable to test article administration.

2. Body Weights - All dogs were weighed weekly throughout the one-year study. Table 2.

Table 2

GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS OF DOGS ADMINISTERED BROMACIL AS A DIETARY ADMIX FOR ONE YEAR

Test Day	Males				Females			
ppm =	0	25	150	625	0	25	150	625
0	9.5	9.3	9.5	9.8	8.4	8.4	8.4	8.7
7	9.5	9.4	9.6	9.8	8.5	8.8	8.6	8.5
14	9.7	9.4	9.7	10.0	8.5	9.0	8.8	8.6
21	9.7	9.3	9.7	9.9	8.6	8.9	8.8	8.7
28	9.9	9.4	9.9	10.0	8.7	9.0	9.0	8.7
56	10.2	9.5	10.0	10.2	9.0	9.3	9.2	8.8
91	10.7	10.0	10.3	10.2	9.5	9.7	9.3	9.0
182	11.3	10.8	10.8	11.5	10.0	10.6	10.3	9.4
273	11.3	11.1	11.0	11.7	10.1	11.0	10.5	9.6
364	11.5	11.1	11.3	12.2	10.5	11.5	11.0	9.8
BODY WEIGHT GAINS (kg)								
0-28	0.4	0.1	0.4	0.2	0.3	0.6	0.6	0.0
0-91	1.2	0.7	0.8	0.4	1.1	1.3	0.9	0.3
0-182	1.8	1.5	1.3	1.7	1.6	2.2	1.9	0.7
0-273	1.8	1.8	1.5	1.9	1.7	2.6	2.1	0.9
0-364	2.0	1.8	1.8	2.4	2.1	3.1	2.6	1.1

There were no statistically significant differences from control at alpha = 0.05.
One male (No. 2905) at 625 ppm sacrificed moribund on day 99.

Data extracted from Report Tables 2-5, pages 67-74.

There appeared to be either no effect or only the slight suggestion of an effect of test article administration on body weights or weight gains in males at 625 ppm. Through day 49, individual control weight gains were 0.3, 0.5, 0.7, 0.9 and 1.2 kg; whereas, in the 625 ppm group, the gains were 0.1, 0.2, 0.3, 0.6 and 1.0 kg.

Through day 105, the control gains were 0.4, 1.3, 1.4, 1.5 and 2.3 kg versus 625 ppm group gains of -0.2, 0.8, 1.5 and 1.6 kg (one dog sacrificed moribund on day 99, started losing weight at the day 63 weighing). From the 6-month interval (DER Table 2 of extracted data), gains in all groups seemed to be equal.

In females, 625 ppm appeared to decrease body weight gain throughout the study (see Table 2 of extracted data). Through day 49, individual control weight gains were 0.0, 0.4, 0.7, 0.7 and 0.8 kg; whereas, in the 625 ppm group, the gains were -0.3, -0.1, 0.1, 0.3 and 0.3 kg. Through day 105, the control gains were -0.2, 1.2, 1.5, 1.7 and 1.9 kg versus 625 ppm group gains of 0.1, 0.2, 0.5, 0.7 and 1.0 kg. At the final weighing on day 364, the individual control gains were 0.7, 1.7, 1.9, 2.9 and 3.2 kg versus 625 ppm group gains of 0.4, 0.8, 0.9, 1.7 and 1.9 kg.

3. Food Consumption - This was measured for each dog during every 7-day period throughout the study. The Report stated that animals were given approximately 350 g of food per day.

In males, the average number of grams of food consumed/dog/day during the 364 days of measurement was essentially equal in the four groups (ppm): 0 = 316, 25 = 341, 150 = 328 and 625 = 317. For females, these values were : 0 = 291, 25 = 294, 150 = 306 and 625 = 258; and therefore, it seems that the female dogs given the 625 ppm diet ate less than did the control or lower (25 or 150 ppm) dosed animals.

The Report (p. 27, Section C.) states "Therefore, the changes in food consumption and food efficiency noted among female dogs of the 625 ppm group are important factors contributing to the decreased body weight gain noted above and can be attributed to the unpalatability of the 625 ppm diet."

4. Test Article Intake (Table 3)

5. Ophthalmological Examinations - All dogs were examined by focal illumination and indirect ophthalmoscopy on day 74 and just prior to terminal sacrifice (excludes one 625 ppm male sacrificed moribund on day 99) by a veterinary ophthalmologist, James M. Clinton, V.M.D. (letters of examination summaries on Report pages 45 and 46).

The ophthalmologist stated that, "... there was no evidence to indicate that the test article, as evaluated in this study, produces ocular changes in Canis familiaris."

Table 3

MEAN DAILY INTAKE OF BROMACIL IN DOGS FED A DIETARY ADMIX FOR ONE-YEAR

ppm	Males	Females
25	0.826a	0.715
150	4.65	4.60
625	17.8	17.3

a = mg/kg/day
Data from Report page 28.

F. Clinical Pathology

Hematology, blood chemistry and urinalysis parameters were examined twice during the 77 day acclimation period (days -75 and -60), as well as at approximately 3, 6, 9 and 12 months after study initiation. [Clinical Pathology Repcrt, Supplemental Report C, page 50, states pre-test days -75 and -64.] The final (12 month) interval was on day 365. Animals were fasted for about 16 hours prior to blood collection (jugular vein) and urine was collected during the fasting period.

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

x	Hematocrit (HCT)*	-	Total plasma protein (TP)
x	Hemoglobin (HGB)*	x	Leukocyte differential count
x	Leukocyte count (WBC)*	x	Mean corpuscular HGB (MCH)
x	Erythrocyte count (RBC)*	x	Mean corpus. HGB conc. (MCHC)
x	Platelet count*	x	Mean corpus. volume (HCV)

x Reticulocyte counts - smears prepared, "evaluation not required"

x Bone marrow smears at sacrifice, "evaluation not required"

* = EPA Guideline Requirement "-" = Not examined

There were no apparent test article effects on any hematological parameter examined. Those group mean values which were statistically significant from controls showed no pattern (dose response or interval) or were within expected normal limits.

NOTE: Male dog No. 2905 (625 ppm), sacrificed moribund on day 99, had the following at the 3 month interval: decrease in erythrocytes, hemoglobin and hematocrit; leukocytosis, neutrophilia, lymphopenia and monocytosis.

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

	Other		Electrolytes
x	Albumin*	x	Calcium*
x	Blood creatinine*	x	Chloride*
x	Blood urea nitrogen*	-	Magnesium*
x	Cholesterol*	x	Phosphorous*
x	Globulin (calculated)	x	Potassium*
x	Glucose*	x	Sodium*
x	Total bilirubin*		
x	Total protein*		
-	Triglycerides		

	Enzymes
x	Alkaline phosphatase
-	Cholinesterase
x	Creatine phosphokinase*
-	Lactic acid dehydrogenase
x	Serum alanine aminotransferase (also SGPT)*
x	Serum aspartate aminotransferase (also SGOT)*

* = EPA Guideline Requirement "-" = Not examined

The administration of BROMACIL to dogs for one year apparently had no effects on any blood chemistry parameters examined.

NOTE: Male dog No. 2905 (625 ppm), sacrificed moribund on day 99, showed increases in alkaline phosphatase and globulin as well as decreases in aspartate aminotransferase, creatine kinase and albumin.

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

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 Requirements "-" = Not examined

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

G. Sacrifice and Pathology

All survivors were sacrificed and necropsied on test days 370-373. Dogs were fasted for at least 16 hours prior to being anesthetized with thiamylal sodium and euthanitized by exsanguination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs were weighed.

Digestive System		Respiratory		Urogenital	
-	Tongue	x	Trachea*	xx	Kidneys*
x	Salivary glands*	x	Lung*	x	Urinary bladder*
x	Esophagus*			xx	Testes*
x	Stomach*		Cardiovasc/ Hematol	x	Epididymides*
x	Duodenum*			x	Prostate*
x	Jejunum*	x	Aorta*	-	Seminal vesicle*
x	Ileum*	xx	Heart*	xx	Ovaries*
x	Cecum*	x	Bone marrow*	x	Uterus*
x	Colon*	x	Lymph nodes*		
x	Rectum*	x	Spleen*		
xx	Liver*	x	Thymus*		
x	Gallbladder*				
x	Pancreas*				

Neurologic		Glandular		Other	
xx	Brain*	xx	Adrenals*	x	Bone*
x	Peripheral nerve*	-	Lacrimal gland	x	Skeletal muscle*
x	Spinal cord (x3)*	x	Mammary gland*	x	Skin*
x	Pituitary*	x	Parathyroids*	x	All gross lesions and masses*
x	Eyes (optic n.)*	xx	Thyroids*		

NOTE: Rib was taken for bone marrow. No evaluation.
x3 = 3 levels
Also taken: tonsil and 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 - %): liver, kidneys, heart, adrenals, brain, thyroid/parathyroid, testes and ovaries.

There were no apparent differences between treated and control values regarding absolute or relative organ weights.

3. Microscopic

Male dog No. 2905 (625 ppm) sacrificed on day 99. The pathologist stated:

> "A spontaneous condition in beagles, variously termed spontaneous disseminated panarteritis[4], periarteritis[5], necrotizing vasculitis[6], polyarteritis[7], and beagel pain syndrome[8], resulted in significant morbidity in one 625 ppm, male dog (2905). The dog was sacrificed after 99 days on test. The lesion, arterial and periarterial inflammation, consisted of varying degrees of arterial mural necrosis and infiltration with inflammatory cells and adjacent, primarily suppurative, cellulitis. It was found in the liver, kidney, left atrium, sciatic nerve, stomach (cardia), mesenteric lymph node, thymus, prostate, urinary bladder, meninges of the spinal cord, and musculature of the cervical vertebrae."

The only microscopic observations which may have been attributed to BROMACIL administration was minimal to mild testicular atrophy and degeneration. The study pathologist stated:

> "Minimal to mild testicular atrophy and degeneration, characterized by decreased germ cell numbers, spermatid giant cell production, or vacuolation of the tubular epithelium, were seen in several treated males (0 ppm = 0/5, 25 ppm = 3/5, 150 ppm = 3/5, 625 ppm = 3/5). This change was more severe and more often bilateral in the 25 ppm group than in the higher treatment groups (150 and 625 ppm), but was minimal to mild in all instances. A low incidence of testicular atrophy has been reported as an incidental finding in laboratory beagles[1,2].

Historical control data also support the occurrence of minimal to mild testicular atrophy as a common spontaneous lesion[3]. This change was not considered compound related."

Table 4

INCIDENCE OF TESTICULAR ATROPHY AND DEGENERATION IN DOGS ADMINISTERED BORAX FOR ONE YEAR AND IN HISTORICAL CONTROLS

Observation ppm =	0	25	150	625
No. dogs with observation	0/5	3/5	3/5	3/5
Atrophy/degeneration, seminiferous tubules, bilateral	0/5	1=minimal 1=mild	0/5	1=minimal
Atrophy/degeneration, seminiferous tubules, unilateral.	0/5	1=mild	3=minimal	2=minimal

HISTORICAL: 6 reports (1986-Mid 1990) - severity was graded minimal or mild (Report page 62).

Bilateral 0/5, 1/5, 0/4, 1/5, 0,5, 0,5
Unilateral 0/5, 1/5, 0/4, 1/5, 1/5, 2/5

A review of the data (control and test article as well as historical) presented in this report, appears to indicate the possibility of test article involvement in testicular atrophy/degeneration. No controls exhibited this finding compared with 3/5 dogs in each dosed group. In addition, the historical data from 6 other studies indicates that, even though the severity is equivalent (minimal or mild), there were 2/6 in which no dogs had this finding, 1/6 in which one dog was involved and 3/6 in which two dogs had the observation.

The Reviewer has no comments to make regarding Materials and Methods.

A detailed description of statistical analyses used in this study was included.

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

II. DISCUSSION

Test article purity, stability and homogeneity data indicated that the dietary concentrations were similar enough to the desired concentrations to be acceptable.

All dogs survived this one year study with the exception of one 625 ppm male which was sacrificed in extremis on day 99 (diagnosis was "spontaneous disseminated panarteritis"). This was not considered to be a result of test article administration. There were no clinical signs attributed to BROMACIL.

There appeared to be a decrease in body weight gain at 625 ppm in males and females. This was particularly noticeable during the first 3 months of the study. The Report gives details regarding the choice of concentrations used in this study and the aspect of "palatability".

Food consumption in males was similar for all groups. The 625 ppm females appeared to eat less (g/dog/day) than did the control or lower concentration groups: 258 versus 291, 294 and 306. This decreased food consumption in females corresponds to the decrease in body weight gain noted in these animals.

There were no apparent test article effects on any parameters regarding hematology, blood chemistry or urinalysis. In addition, no ophthalmic, gross necropsy or organ weight findings were attributed to BROMACIL administration.

The presence of unilateral or bilateral testicular atrophy/ degeneration in 3/5 dogs in each of the three treatment groups plus the included historical data from six studies, presents the possibility that not only may this finding be a test article effect, but that, as even the lowest concentration tested had animals involved, there may not be a No Observed Effect Level (NOEL) in this study. In consultation with Veterinary Pathologist Lucas Breneke, DVM, PhD, ACVP, the data presented suggest that this chemical may have a testicular effect.

III. CONCLUSIONS

BROMACIL, administered by dietary admix at concentrations of 0, 25, 150 and 625 ppm to dogs for one year appeared to cause a slight decrease in body weight gain in males and especially in females at 625 ppm. In addition, there is the suggestion that all three concentrations may increase the incidence to testicular atrophy/degeneration.

The No Observed Effect Level (NOEL) = <25 ppm (0.625 mg/kg), lowest dose tested - suggestion of possible testicular atrophy/ degeneration.

The Lowest Observed Effect Level (LOEL) = 25 ppm, lowest dose tested - testicular atrophy and degeneration.

Classification: **Core Supplementary.** This is due to testicular observations reported at all concentrations tested. The Registrant may wish to present to the Agency additional data to substantiate that this finding (at this incidence - 3/5 in each group versus 0/5 in control) is not likely to have been the result of BROMACIL administration.

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