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

NOV - 6 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of a Chronic Toxicity Study in Dogs with Polyhexamethylene Biguanide (PHMB).

EPA Identification Numbers:
DP Barcode: D214692 MRID #'s 43620501
P.C. Code: 111801 Submission: S486009

TO: Bruce Sidwell / Marie Boucher
Product Manager # 53
Special Review and Reregistration Division (7508W)

FROM: Timothy F. McMahon, Ph.D. 9/28/96
Pharmacologist, Review Section I
Toxicology Branch II, Health Effects Division (7509C)

THRU: Jess C. Rowland, M.S. 10/24/96
Acting Section Head, Review Section I
Toxicology Branch II, Health Effects Division (7509C)

and

Yiannakis M. Ioannou, Ph.D. 11/6/96
Acting Chief, Toxicology Branch II
Health Effects Division (7509C)

Registrant: Zeneca Ag Products

Action Requested: Review of a chronic toxicity study conducted with PHMB in the dog.

1/22

Recommendations: Toxicology Branch II has reviewed the chronic toxicity study in the dog (MRID # 43620501) and has determined that the study is **acceptable** and satisfies the guideline requirement (OPPTS 870.4100, OPP §83-1) for a chronic toxicity study in non-rodents. The executive summary is presented below.

Executive Summary

In a chronic toxicity study (MRID # 43620501), polyhexamethylene biguanide was administered to groups of 4 male and female Beagle dogs in the diet initially at dose levels of 0, 300, 1500, and 4500 ppm (0, 7.5, 37.5, and 112.5 mg/kg/day nominal dose) for one year. Following an unexpectedly severe reaction in 3 of 4 males at 4500 ppm (scrotal skin lesions), the high dose was discontinued on week 9 or 10, reduced to 3000 ppm (75 mg/kg/day), and then recommenced on week 11 or 12. Up to and including the 3000 ppm dose, there were no consistent effects of PHMB on body weight, weight gain, food consumption, or hematological parameters. Plasma alanine aminotransferase (ALT) activity was significantly increased in male and female dogs at the 3000 ppm dose level beginning at week 8, but there was variability in the response, and only one male dog was available for measurement after week 10. Testes weight was decreased 29% and 32% for the left and right testis of high dose male dogs, and testicular tubular degeneration was observed in the surviving male dog as well as in one dog sacrificed intercurrently. Liver weight in high dose male dogs was decreased 14% at the high dose, and microscopic changes of the liver were also observed in male dogs at the high dose. In one female dog at the high dose, significant clinical signs (decreased activity, stiff/splayed gait, slight tremors) were observed which were not reversible. In addition, plasma alanine aminotransferase was increased almost 10-fold over the pre-treatment level by week 35 of treatment. Plasma aspartate aminotransferase in this dog was almost doubled by week 35 of treamtent. Marked dermatitis of the limbs was also observed in this dog. **The LOEL is 3000 ppm (75 mg/kg/day) for male and female dogs,** based on changes in testis and liver weight and microscopic observations in male dogs, and based on clincal signs of toxicity and clinical chemistry alterations in the female dog. **The NOEL is 1500 ppm (37.5 mg/kg/day) for male and female dogs.**

This study is classified as **acceptable** and satisfies the guideline requirement [OPPTS 870.4100; OPP § 83-1] for a chronic oral toxicity study in dogs.

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EPA Reviewer:Timothy F. McMahon, Ph.D. _____ Date: 8/27/96
Section I, Toxicology Branch II (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 8/27/96 Date: _____
Section I, Toxicology Branch II (7509C)

Data Evaluation Record

Study type: Chronic Oral Toxicity - dogs; OPPTS 870.4100 [§83-1b]. Guideline: §83-1

DP Barcode: D214692
P.C. Code: 111801

Submission: S486009
Tox. Chem. No.:

Test material: Polyhexamethylene biguanide, 20.2% a.i.

Synonyms: Baquacil

Citation: Horner, S.A. (1995): Polyhexamethylene Biguanide: 1 Year Dietary Toxicity Study in Dogs. Zeneca Central Toxicology Laboratory, Cheshire, UK. Laboratory Report No. CTL/P/4488; Study No. PD0947. MRID # 43620501.

Sponsor: Zeneca Biocides, Wilmington, Delaware.

Executive Summary:
In a chronic toxicity study (MRID # 43620501), polyhexamethylene biguanide was administered to groups of 4 male and female Beagle dogs in the diet initially at dose levels of 0, 300, 1500, and 4500 ppm (0, 7.5, 37.5, and 112.5 mg/kg/day nominal dose) for one year. Following an unexpectedly severe reaction in 3 of 4 males at 4500 ppm (scrotal skin lesions), the high dose was discontinued on week 9 or 10, reduced to 3000 ppm (75 mg/kg/day), and then recommenced on week 11 or 12. Up to and including the 3000 ppm dose, there were no consistent effects of PHMB on body weight, weight gain, food consumption, or hematological parameters. Plasma alanine aminotransferase (ALT) activity was significantly increased in male and female dogs at the 3000 ppm dose level beginning at week 8, but there was variability in the response, and only one male dog was available for measurement after week 10. Testes weight was decreased 29% and 32% for the left and right testis of high dose male dogs, and testicular tubular degeneration was observed in the surviving male dog as well as in one dog sacrificed intercurrently. Liver weight in high dose male dogs was decreased 14% at the high dose, and microscopic changes of the liver were also observed in male dogs at the high dose. In one female dog at the high dose, significant clinical signs (decreased activity, stiff/splayed gait, slight tremors) were observed which were not reversible. In addition, plasma alanine aminotransferase was increased almost 10-fold over the pre-treatment level by week 35 of treatment. Plasma aspartate aminotransferase in this dog was almost doubled by week 35 of treamtent. Marked dermatitis of the limbs was also observed in

this dog. **The LOEL is 3000 ppm (75 mg/kg/day) for male and female dogs,** based on changes in testis and liver weight and microscopic observations in male dogs, and based on clincal signs of toxicity and clinical chemistry alterations in the female dog. **The NOEL is 1500 ppm (37.5 mg/kg/day) for male and female dogs.**

This study is acceptable and satisfies the guideline requirement [OPPTS 870.4100; OPP § 83-1] for a chronic oral toxicity study in dogs

COMPLIANCE : Signed and dated statements of Good Laboratory Practice, Quality Assurance, and EPA Flagging were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Polyhexamethylene biguanide (PHMB).
Description: very faint yellow liquid
Lot/Batch # D4097; CTL reference # Y00156/008.
Purity: 20.2% w/w
Stability: stable under the conditions of storage and for the duration of the study (Appendix B, page 186 of the report).

2. Vehicle: dietary preparation

3. Test Animals: Male and female beagle dogs. Source: colony maintained at Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK. Acclimation period: 4 to 5 weeks at the CTL doghouse. Age: 17-20 weeks. Weight range: 9.6-14.4 kg (males); 8.4-11.5 kg (females). Dogs were vaccinated against canine viral hepatitis, distemper, leptospirosis, and canine parvovirus while at the breeding facility. Dogs also received regular treatment for possible nematode and ear mite infestation. Upon arrival at the testing laboratory, dogs were housed in indoor pens in groups of two or three (sexes separate) for at least seven days. Thereafter, dogs were housed individually in pens measuring 365 x 115 cm which consisted of sleeping quarters with a heated floor and a separate exercise area. Temperature was between 18-24 ^{o}C with a 12 hour light/dark cycle and approximately 10 air changes per hour. Male dogs received 400g and females 350g of LABORATORY DIET A (Special Diets Services Ltd, Essex, UK) each morning.

B. STUDY DESIGN

1. In life dates: May 1993-May 1994

2. Animal Assignment

The study consisted of 4 males and 4 females per dose group, assigned to the following groups:

Test Group	Dose (ppm)	Experimental Animals	
		Males	Females
1	0	701-704	705-708
2	300	709-712	713-716
3	1500	717-720	721-724
4	3000	725-728	729-732

The study was conducted with two randomized blocks, each comprised of two male and two female replicates.

3. Dose Selection Rationale: There was no stated rationale for dose selection in this study. However, it was stated (page 16 of the report) that the high dose group was originally dosed with 4500 ppm of PHMB. All high dose animals were removed from this dose level at week 9 or 10 (depending on the replicate), due to the unexpected death of one male dog, clinical signs of toxicity (scrotal skin lesions), and/or inappetance and body weight loss in other dogs at this dose level. Dogs were resumed with treatment on week 11 or 12. As the high dose dogs received the 3000 ppm dose during the majority of the study, the high dose is referred to as 3000 ppm in this study.

4. Diet Preparation and Analysis

Experimental diets were prepared in 60kg batches from a number of pre-mixes. Each pre-mix was prepared by adding the appropriate amount of PHMB to 1kg of Laboratory Diet A. Pre-mixes were then added to the required amount of Laboratory Diet A, to make up a total of 60 kg, and mixed thoroughly. All prepared diets were then pelleted.

It is noted in this report that "due to extreme difficulty in extracting the compound from the diet, the measurement of achieved concentration, homogeneity, and stability was not performed. Homogeneity of the mixing procedure was determined by analyzing the distribution of an aqueous solution of an ionically similar dyestuff, methyl violet." The procedure stated here is consistent with the conclusions reached from a meeting between the registrant and representatives of Health Effects Division on May 21, 1992 (memorandum from Timothy F. McMahon to Linda Deluise, Special Review and Reregistration, dated July 22, 1992). Therefore, the registrant's method of dietary analysis is acceptable to the Agency.

5. Statistics

Body weights were considered by analysis of covariance on initial (day 1) body weight. Hematology and clinical chemistry data were analyzed by analysis of covariance on pre-experimental values. Urinalysis data was analyzed by analysis of variance at each time point of sampling. Male and female data were analyzed together and the results examined to determine whether any differences between control and treated groups were consistent between sexes. Organ weights were considered by analysis of variance and analysis of covariance on final body weight.

C. METHODS

1. Observations

Dogs were observed at least twice daily for clinical or behavioral abnormalities (after dosing and at the end of the working day). Dogs were given a thorough examination on a weekly basis. A daily record of fecal consistency was made. A full clinical examination was conducted by a veterinarian pre-study, and again on weeks 13, 26, 39, and prior to termination. The examination included cardiac and pulmonary auscultation and indirect ophthalmoscopy.

2. Body Weight

Dogs were weighed weekly.

3. Food Consumption and Compound Intake

Food residues were recorded daily prior to giving the next meal and any residual food discarded. These measurements were made for at least 2 weeks pre-study and throughout the treatment period.

4. Ophthalmoscopic Examination

As mentioned, ophthalmoscopic examination was made during the full clinical examinations conducted pre-study and on weeks 13, 26, 39, and prior to termination.

5. Hematology

Jugular vein samples were taken prior to feeding on weeks -1, 4, 13, 26, and 52 (prior to termination). Samples were also taken from intercurrent animals 728, 725, 726, and 732 on weeks 9, 15, 15, and 35, respectively. The following parameters were examined:

_x_ total leucocyte count*	
_x_ erythrocyte count*	_x_ leukocyte differential*
_x_ hemoglobin (HGB)*	_x_ mean corpuscular HGB
_x_ hematocrit (HCT)*	_x_ mean corpusc. HGB conc.
_x_ platelet count	_x_ mean corpusc. volume
_-_ packed cell volume	_-_ methemoglobin
_-_ reticulocyte count	_-_ partial thromboplastin time
	_-_ fibrinogen
	_x_ kaolin-cephalin time
	_x_ prothrombin time

*EPA guideline requirement "-" not analyzed

6. Clinical Chemistry

The following parameters were examined:

_x_ glucose*
_x_ albumin*
_-_ A/G ratio (calculated)
_x_ creatinine
_x_ total bilirubin*
_-_ direct bilirubin
_-_ indirect bilirubin
_x_ urea nitrogen*
_x_ total protein*
_x_ cholesterol*
_x_ triglycerides
_-_ electrophoretic protein fractions

_x_ AST(SGPT)*
_x_ ALT(SGOT)*
_x_ alkaline phosphatase
_x_ creatine kinase*
_-_ lactate dehydrogenase
_-_ sorbitol dehydrogenase
_x_ gamma glutamyl trans-peptidase
_-_ ornithine carbamyl transferase
_-_ 2,3-diphosphoglyceric acid
_-_ plasma ChE
_-_ red cell ChE
_-_ urea

_x_ calcium*
_x_ inorganic phosphate*
_x_ sodium*
_x_ potassium*
_x_ chloride*

{*EPA guideline requirement; "-" not examined}

According to the report (page 18), additional blood samples were taken in weeks 8, 17, 21, and 42 from all dogs, and in week 10 from dogs in replicates 1, 2, 5, and 6, for measurement of one or more of the following: plasma alanine transaminase, aspartate transaminase, creatine kinase, and alkaline phosphatase.

7. Urinalysis

Urine samples were obtained by catheterization pre-experimentally, and again on weeks 26 and 52 (prior to termination). The following parameters were measured:

_x_ volume
_x_ pH
_x_ specific gravity
_x_ protein
_x_ glucose
_x_ bilirubin
_x_ urobilinogen
_x_ ketones
_x_ blood

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8. Sacrifice and Pathology

All dogs surviving to termination and those sacrificed intercurrently were anesthetized by i.v. sodium pentobarbitone injection. Dogs were then killed by exsanguination and subjected to a full necropsy. The weight of the adrenal glands, brain, epididymides, kidneys, liver, testes, and thyroid glands was taken. Left and right components of paired organs were weighed separately. The following organs were collected for histological examination. The (XX) organs, in addition, were weighed.

Digestive	Respiratory	Urogenital
- tongue	x trachea	xx kidneys*
x salivary glands*	x lungs*	x urinary bladder*
x esophagus*	- nasal cavity	xx testes*
x stomach*		xx epididymides*
x duodenum*	Cardiovascular	- seminal vesicle*
x jejunum*	x aorta*	x prostate
x ileum*	x heart*	x ovaries
x cecum*	x bone marrow	x uterus*
x colon*	x lymph nodes*	x vagina
x rectum*	x spleen*	x cervix
xx liver*		
x pancreas*		
x gall bladder*		

Neurologic	Glandular	Other
xx brain*	xx adrenals*	x bone (femur)
x peripheral nerve*	- lacrimal gland	x skeletal muscle
x spinal cord *	x mammary gland	x skin*
x pituitary*	x parathyroids*	x all gross lesions*
x eyes	xx thyroids*	x scrotal skin
	x thymus*	

*EPA guideline requirement "-" not examined

II. **RESULTS**

A. Observations

1. **Mortality** - During the study, 3 of 4 high dose males and one of 4 high dose females were killed for humane reasons during the study. One male was killed on week 9 after receiving PHMB at 4500 ppm. Two males were killed on week 15 after receiving 4500 ppm PHMB from weeks 1-10 and 3000 ppm PHMB from week 12. For males, reason(s) for sacrifice were reddening and peeling of the scrotal skin (all sacrificed males), inappetance (1 male), body weight loss (1 male), and marked increase in plasma alanine transaminase and aspartate transaminase activities (1 male). The female dog was sacrificed on week 35 after receiving 4500 ppm PHMB for weeks 1-10 and 3000 ppm PHMB since week 12. This dog showed decreased activity, stiff/splayed gait, and slight tremors, from which recovery was not apparent. In addition, body weight loss, peeling of the skin on the pads of the paws, staining of paws and hocks, forelimb, hindlimb, and forepaw abrasions, scabs, and sores, and elevated plasma alanine transaminase and aspartate transaminase activities were also evident in this dog.

2. **Toxicity** - The following observations were noted for male dogs in this study:

	Dose (ppm)			
	0	300	1500	3000
forelimb abrasions	0/4	0/4	1/4	2/4
week(s) observed	-	-	44	13-47
scrotal skin peeling	0/4	1/4	0/4	4/4
week(s) observed	-	42-43	-	3-10
scrotum reddened	1/4	2/4	2/4	4/4
week(s) observed	41	14-48	18-53	2-15

Data obtained from pages 45-48 of the report.

According to the report, one of the high dose male dogs (number 727) was observed with reddening and peeling of the scrotal skin during weeks 1-13. This was particularly marked, and required veterinary treatment. This occurred during the time that the high dose was 4500 ppm instead of 3000 ppm. The report also stated that the scrotal skin reddening and peeling observed at the lower doses were not as marked as that which occurred at the high dose. Staining/discoloration of the coat was observed in one 300 ppm group female and all surviving animals in the 1500 and 3000 ppm groups.

The report also noted that inflammation/reddening of the ear canal and/or pinna with large amounts of dark or brown fluid discharge, was frequently observed for one male and one female receiving 1500 ppm throughout the study, often requiring veterinary treatment. Reddening of the ears and/or dirty

ears were also seen on occasion for one control male, for one male and two females at 300 ppm, for one additional male at 1500 ppm, and for 2 females at 3000 ppm, but were not associated with a discharge.

Distension of the abdomen was observed for one male and one female at 300 ppm throughout the majority of the study and on one occasion only for one female at 1500 ppm. This was observed usually several hours after feeding with full recovery occurring prior to feeding on the following day.

B. Body Weight

A summary of body weight effects is shown in the following Tables for male and female dogs:

TABLE 1a

Group Mean Body Weights (in kg) in Male Dogs Given PHMB in the Diet for 52 Weeks[a]

Week of Study	Males (ppm) 0	300	1500	3000
N	4	4	4	4
1	11.50± 1.95	11.30±0.82	11.60±0.88	11.55±1.24
7	12.88±1.37	12.73±0.49	13.08±1.04	12.55±1.20
13	13.55±0.91	13.45±0.40	13.70±1.07	13.30±0.46 (N = 3)
26	14.18±0.39	14.20±0.42	14.13±1.06	13.60 (N = 1)
53	14.60±0.50	14.98±0.71	14.30±1.19	13.50 (N = 1)

[a]data taken from pages 69-75 of the report.

TABLE 1b

Group Mean Body Weights (in kg) in Female Dogs Given PHMB in the Diet for 52 Weeks[a]

Week of Study	Females (ppm) 0	300	1500	3000
N	4	4	4	4
1	10.20± 1.51	10.28±1.43	10.20±0.59	9.60±0.80
7	10.85±1.53	11.18±1.44	11.38±0.51	10.00±0.80
13	11.20±1.59	11.75±1.27	12.05±0.65	10.43±0.35
26	11.55±1.85	12.05±1.18	12.50±0.76	11.20±0.45
52	12.05±2.09	12.80±1.27	13.28±1.28	12.10±0.66

[a]data taken from pages 76-82 of the report.

As shown in the above tables, there were no apparent treatment-related effects on group mean body weight in male or female dogs at any dose level tested. Changes in group mean body weight gain are summarized below for weeks 1-13 and 0-52 (calculated from individual body weight data, pages 297-328 of the report):

TABLE 2a

Group Mean Body Weight Gain in Male Dogs Given PHMB in the Diet for 52 Weeks

	Males (ppm) 0	300	1500	3000
Weight gain (kg):				
1-13	2.05	2.15	2.10	1.16 (N=3)
% control	-	105	102	56
1-52	3.05	3.52	2.60	no data
% control	-	115	85	-

[a]data calculated from the report.

In male dogs, body weight gain for weeks 1-13 was affected at the high dose, where dogs in this group showed a decrease of 44% from control in group mean weight gain. For the duration of the study, a decrease in weight gain of 15% was also observed at the mid dose. It is noted that in the high dose group, there was only one male dog surviving to study termination beyond week 16 of the study.

TABLE 2b

Group Mean Body Weight Gain in Female Dogs Given PHMB in the Diet for 52 Weeks

Females (ppm)

Weight gain (kg):	0	300	1500	3000
1-13	1.0	1.47	1.85	0.83
% control	-	147	185	83
1-52	1.85	2.52	3.08	2.50 (N=3)
%control	-	136	166	135

[a]data calculated from the report.

Similar to male dogs, decreased body weight gain (17%) was observed in female dogs at the high dose for weeks 1-13. For the study duration, body weight gain was not significantly decreased at any dose level in female dogs.

C. Food Consumption and Efficiency

1. Food Consumption

The results of food residue measurement (pages 294-295 of the report) are not consistent with the statement in the report (page 18) that "food residues were... (recorded) at least 2 weeks pre-study and throughout the study period." The results as presented show only occasional measurements, and cannot be correlated with any changes in body weight gain. According to the report (page 24), "food consumption was essentially similar to that of controls" following reduction of the high dose to 3000 ppm. This statement cannot be verified from the available data.

2. Intake of PHMB

In the absence of data showing actual intake of the test chemical, and based on the data in the report illustrating acceptable homogeneity based on the mixing procedure, the intake of test chemical can be considered as nominal. Therefore, for the 0, 300, 1500, and 3000 ppm dose groups, the intake can be stated as **0, 7.5, 37.5, and 75 mg/kg/day.**

D. Ophthalmoscopic Examination

Based on the inclusion of indirect ophthalmoscopy in the clinical examination and the lack of reporting of any abnormalities in the clinical observation section, it appears that treatment with PHMB caused no significant ocular effects in male or female dogs. Data reporting negative findings from eye examinations would have been useful in supporting this conclusion; the reviewer's conclusion is based only on the lack of any statements to the contrary in the report.

E. Blood Work

1. Hematology

The results of hematological measurements were presented on pages 85-112 of the report (Table 8). There did not appear to be consistent changes in hematology in male or female dogs over the course of treatment with test chemical, but there were some changes that can be summarized as follows:

a) Red cell count in female dogs was observed to be decreased by 4, 9, 3, and 11% at the 3000 ppm dose for weeks 4, 13, 26, and 52, respectively, vs concurrent control.

b) Mean cell volume was decreased by 13% at week 13 for male dogs at the high dose vs concurrent control.

c) Platelets were increased by 21% at week 13 for high dose male dogs, and by 33% at week 52 for high dose male dogs. High dose female dogs showed an increase in platelets of 15% at week 13, and an increase of 8% at week 26 at the high dose.

d) Eosinophils in high dose male dogs were decreased by 39% at week 4, and 34% at week 13. In females, eosinophil counts were elevated at these time points.

From these observations, it is concluded that while there appear to be some effects of treatment at the 3000 ppm dose level on hematology parameters, these effects are not consistent over the course of the treatment period and are transient in nature.

2. Clinical Chemistry

Clinical chemistry data were summarized on pages 113-150 of the report. The report noted effects observed in those dogs sacrificed intercurrently as well as in those surviving to study termination. For those dogs sacrificed intercurrently (males # 728, 725, and 726; female # 732), marked increases in plasma alanine and aspartate transaminase activity were noted. For the sacrificed male dogs, the maximum increase in alanine and aspartate transaminase was noted to have occurred around weeks 10-13. This was also the time during which the high dose was 4500 ppm. In the sacrificed female dog, the maximum increase in transaminase activities was noted at week 26.

A summary of effects noted in those dogs surviving to study termination is summarized below (Table 3):

TABLE 3

Blood Chemistry in Male and Female Dogs Given PHMB Technical in the Diet for 52 Weeks

	Dose (ppm)			
	0	300	1500	3000
N =	4	4	4	4(F) 3(M)
Week 8				
alanine aminotransferase (IU /L)				
males	27.3±13.1	24.0±8.1	32.8±8.6	233.5±391.7 (N=4)
females	20.0+2.2	18.5±5.4	27.5±7.3	95.8±123.6
Week 10 (N=2, M and F)				
alanine aminotransferase (IU /L)				
males	22.5±6.4	22.5±2.1	25.0±1.4	70.5±27.6
females	22.0+4.2	20.0±1.4	23.5±9.2	335.0±376.2

Table 3, cont.

	Dose (ppm)			
	0	300	1500	3000
N =	4	4	4	4(F) 3(M)
Week 13				
cholesterol (mg / 100ml)				
males	149±24	137±33	148±13	109±7
females	186±46	179±60	147±25	125±20
total bilirubin (µmol/l)				
males	0.2±0.0	0.18±0.05	0.2±0.0	0.1±0.0
females	0.25±0.06	0.25±0.06	0.2±0.0*	0.2±0.0
alkaline phosphatase (IU /L)				
males	145±61	141±46	152±34	109±55
females	172±24	161±21	150±22	137±42*
alanine aminotransferase (IU /L)				
males	26.3±6.8	27.5±10.2	37.8±11.0	53.3±14.5
females	25.0+5.1	24.3±11.0	35.5±20.4	54.3±10.4

Table 3, cont.

	Dose (ppm)			
	0	300	1500	3000
N =	4	4	4	4(F) 1(M)
Week 26				
cholesterol (mmol/l)				
males	147±20	138±34	128±15	95
females	196±39	176±66	138±10*	105±15*
alanine aminotransferase (IU /L)				
males	33.8+11.2	27.5±4.4	41.0±11.8	90.0
females	27.5±12.4	32.8±30.9	48.0±34.0	101.3±69.5
Week 52				
cholesterol (mmol/l)				
males	131±12	130±25	126±5	118
females	174±28	174±64	164±25	119±20**
alanine aminotransferase (IU /L)				
males	41.8+15.6	26.8±6.9	40.0±8.8	69.0
females	29.8±6.2	40.0±38.7	72.5±87.2	61.3±35.7 (N=3)

[a] data from pages 113-150 of the report. * $p < 0.05$ vs control. ** $p < 0.01$ vs control.

An effect noted early in the study (week 8) at the top dose level in both male and female dogs, and one of the earliest effects measured, was the increase in alanine aminotransferase activity. At week 8 of the study, the activity of this enzyme was increased between 3 and 15-fold in male and female dogs. By week 13, the increase was still apparent in both sexes, but was not as great (approximately 2-fold). Subsequent measurements (weeks 26 and 52) showed increases of approximately 2- to 3-fold in the activity of this enzyme at the 3000 ppm dose level in both male and female dogs, although it is noted that there was only one male dog who survived for the week 26 and 52 measurement. It should also be noted that at the 300 ppm dose level, one female dog (number 715) showed increases in alanine aminotransferase activity between 2 and 3-fold over control beginning at week 17 and continuing to the end of the study. An additional female dog (number 723) at the 1500 ppm dose level also showed an increase in the activity of this enzyme between 2-4 fold over control at week 13 and continuing until study termination.

Other effects noted on clinical chemistry included: a decrease in plasma cholesterol in male and female dogs at the 3000 ppm dose level at week 13 (27% decrease in males, 33% decrease in females), week 26 (36% decrease in males, 47% decrease in females), and week 52 (10% decrease in males, 32% decrease in females); and decreased total bilirubin at week 13 in males and females.

F. Urinalysis

The results of urinalysis were presented in Tables 10A and 10B of the report, pages 152-160 of the report. These data showed no significant changes in urinary clinical chemistry or urine cytology in male or female dogs at any dose level tested.

G. Sacrifice and Pathology

1. Organ Weights

Organ weight data were summarized for male and female dogs on pages 161-162 of the report. There were no significant organ weight changes for female dogs in this study. In male dogs, there were changes in some organ weights at the 3000 ppm dose level, but interpretation is hindered by only one surviving dog at this dose. The report stated that for those dogs killed during the study, there were no significant organ weight changes to report. In the one surviving male dog, the weight of the adrenals was increased from 1.30±0.12 grams in control to 1.74 grams at the high dose. The weight of the epididymides was decreased from 4.86±0.93 grams in control to 3.75 grams at the high dose. The weight of the testes were markedly decreased, from 21.6±3.9 grams in control to 7.9 grams at the high dose. Kidney weight was

increased from 55.8±8.4 grams in control to 87.2 grams at the high dose, while liver weight appeared decreased, from 401±18 grams in control to 317 grams at the high dose.

2. Gross Pathology

A summary of gross pathology reported for those dogs sacrificed during the study was presented on pages 163-164 of the report. One high dose male was noted with an accentuated lobular pattern of the liver and scaly areas of the scrotum. In another sacrificed male dog, reddening of the scrotal skin with multiple scabs was noted, as was firm nodules of the lung, enlargement of the prescapular lymph nodes, and hair loss and thickening of the skin of the lower limbs. The third sacrificed male was noted with severe scrotal lesions, scabs on the scrotum and pale lungs.

Gross pathology in those dogs surviving to study termination was shown on pages 165-169 of the report. Again, definitive interpretation is hindered by only one surviving male dog at the high dose. However, the findings are summarized according to dose level as follows:

3000 ppm

Pale areas of the gastrointestinal tract (cecum, colon, duodenum, ileum, jejunum) and reproductive tract (cervix, uterus) in one high dose female dog).

A small polypoid mass in the oral cavity of one high dose female dog, and a firm pale mass in the oral cavity of the surviving high dose male dog.

Reduced testis size of 50% and red medulla in the kidney of the surviving high dose male dog.

Small skin mass on the right hind limb of one female dog.

Discolored hair on the lower limbs of three female dogs.

1500 ppm

One male dog observed with depressed areas of the tongue and red areas on the scrotum; another observed with cecal intussusception.

Unilateral abnormality of the epididymis, in which the epididymis was reduced to a thin strand of tissue and the caput was discolored yellow.

Discolored hair on the lower limbs of three male dogs.

300 ppm

Discolored hair on the lower limbs of one female dog.

3. Microscopic Observations

For those dogs sacrificed during the study (as a result of the 4500 ppm dose administration), the following microscopic pathologic changes were noted (incidence at lower doses was zero in all cases) [Table 14, pages 171-172]:

KIDNEY

- Intratubular microlithiasis in 2 of 3 male dogs, and 1 female dog.
- Increased cortical tubular hyaline droplet formation in 3 of 3 male dogs.

LIVER

- Cellular swelling in 3 of 3 male dogs, and 1 female dog.
- Hepatocyte pigmentation (minimal to slight) in 3 of 3 male dogs, and 1 female dog.
- Macrophage/Kuppfer cell pigmentation (minimal to slight) in 3 of 3 male dogs, and 1 female dog.
- Hepatocellular eosinophilic intracytoplasmic inclusion bodies (slight) in 3 of 3 male dogs and 1 female dog. Single cell necrosis also observed in 1 of 3 male dogs.

SKIN

- Marked dermatitis of the limbs in 1 of 3 male dogs and 1 female dog.
- Dermatitis of the scrotum in 2 of 3 male dogs; 1 slight and 1 marked.

TESTES

- Bilateral tubular degeneration (slight) in 1 of 3 male dogs.

The following microscopic observations were recorded in dogs surviving to study termination 3000 ppm [Table 14, pages 173-182]:

- Hepatocellular eosinophilic intracytoplasmic inclusion bodies (slight) in the surviving male dog and in 3 of 3 surviving female dogs.
- Mineralized foci of spinal nerves in the surviving male dog and in 1 of 3 surviving female dogs (also seen in one control female dog).
- Bilateral tubular degeneration of the testes (marked) in the surviving male dog.

The report commented that the pale areas of the gastrointestinal tract and uterus in the female dogs sacrificed during the study had no microscopic counterpart. The oral mass observed in this female dog was determined to be a papilloma, a common lesion of viral origin in young dogs. The oral mass in the male dog was connective tissue covered with a normal epithelium. The depressed areas of the tongue and the cecal intussusception were considered of no toxicological significance. The report stated that the grossly reduced epididymides of the two male dogs were associated with sperm granulomata, lesions which occur spontaneously in most species. However, the report also stated (page 35) that the "bilateral testicular tubular degeneration...was considered to be related to treatment with PHMB." Whether this refers to the same lesion is not clear.

III. DISCUSSION

In the present study, groups of 4 male and 4 female beagle dogs were assigned to receive the test article, polyhexamethylene biguanide (PHMB) in the diet at doses of 0, 300, 1500, and 4500 ppm. Due to excessive toxicity noted at the 4500 ppm dose level after approximately 9 weeks (death/sacrifice of 3 of 4 male dogs, 1 of 4 female dogs; severe scrotal lesions in male dogs), the dose level was decreased to 3000 ppm for the duration of the study. Up to and including the 3000 ppm dose level, there were no consistent effects on body weight, weight gain, food consumption, or hematology. Clinical chemistry measurements showed that early in the study (week 8 and subsequent times), the activity of alanine aminotransferase was markedly increased in male and female dogs. The large standard deviation observed for both sexes at week 8 is based on the observation of a dramatic increase in the activity of this enzyme in one dog of each sex (for males, dog # 728 with an activity of 821 IU/L compared to 37, 42, and 34 IU/L for the other three; for females, dog # 730 with an activity of 281 IU/L compared to 40, 32, and 30 IU/L for the other three). At week 13 and subsequent times of measurement, increases in alanine aminotransferase were still evident, and standard deviations were lower. However, only one male dog was available after week 13 at the 3000 ppm dose, making interpretation of the effect difficult. In addition, one female dog each at the 1500 and 300 ppm dose levels showed increases in alanine aminotransferase activity at weeks 13 and 17,

cannot be ruled out, as activity subsequent to lowering the dose tended to be decreased in comparison to the first 13 weeks.

The organ weight change observed in the testes of male dogs at the high dose is of interest in light of the scrotal lesions observed at the high dose(s) tested. In control male dogs, mean left and right testis weights were stated as 10.68 grams and 10.89 grams, respectively, while at the 3000 ppm dose, mean left and right testis weights were stated as 7.66 and 7.46 grams, a decrease of 29% and 32%, respectively. Two male dogs at the high dose were also observed with bilateral testicular tubular degeneration unaccompanied by inflammation. The organ weight changes and microscopic changes of the testes could be related to test article administration. In addition, liver weight in high dose male dogs was decreased from 401 grams to 348 grams, a decrease of 14%. The liver of high dose male dogs was also observed with cellular swelling, hepatocyte pigmentation (minimal to slight), hepatocellular eosinophilic intracytoplasmic inclusion bodies (slight), and single cell necrosis (1 dog). It appears that changes in testes weight and liver weight in males were associated with microscopic changes at the 3000 ppm dose level.

Based on the changes in testis weight and the testicular tubular degeneration observed in male dogs, the Lowest Observed Effect Level (LOEL) is considered to be 3000 ppm (75 mg/kg/day). For female dogs, the Lowest Observed Effect Level is considered to be 3000 ppm, based on the significant clinical toxicity and alterations in clinical chemistry observed in female dog # 732. The 3000 ppm dose can be considered as an effect level for male dogs, for although only one male dog survived to study termination, the changes in testis weight and microscopic changes were observed in the dogs which were sacrificed intercurrently as well as in the dog which survived to termination, supporting the hypothesis that such effects would have been present if the other dogs had survived. The No Observed Effect Level is considered to be 1500 ppm (37.5 mg/kg/day) for male and female dogs.

Classification

This study is classified as **acceptable** and satisfies the guideline requirement (OPPTS 870.4100; OPP §83-1) for a chronic toxicity study in dogs.