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

DEC - 2 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of a 2-Generation Reproduction Study in Rats with Polyhexamethylene Biguianide (PHMB).

EPA Identification Numbers:
DP Barcode: D214646 MRID # 43617401
P.C. Code: 111801 Submission: S485943

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

FROM: Timothy F. McMahon, Ph.D. 9/5/96
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. 11/26/96
Acting Chief, Toxicology Branch II
Health Effects Division (7509C)

Registrant: Zeneca Ag Products

Action Requested: Review of a 2-generation reproduction study conducted with PHMB in the rat.

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Recommendations: Toxicology Branch II has reviewed the 2-generation reproduction study in the rat (MRID # 43617401) and has determined that the study is **acceptable** and satisfies the guideline requirement (OPPTS 870.3800, OPP §83-4) for a 2-generation reproduction study in rodents. The executive summary is presented below.

Executive Summary

In a multigeneration reproduction study (MRID # 43617401), male and female Alpk:APfSD rats (26 males/dose; 26 females/dose), obtained from the Barriered Animal Breeding Unit at Zeneca Pharmaceuticals, Alderley Park, UK, received PHMB technical (20.2% a.i.) in the diet at nominal doses of 0, 200, 600, and 2000 ppm (23.0, 69.6, and 238.9 mg/kg/day for F_0 males; 25.3, 77.0, and 258.2 mg/kg/day for F_0 females; 23.9, 71.3, and 249.3 mg/kg/day for F_1 males; and 26.1, 79.2, 270.5 mg/kg/day for F_0 females). The rats in each generation received test diets continuously until termination. Systemic toxicity was observed at the 2000 ppm dose level in the F_0 generation as indicated by a decrease in group mean body weight (9-10%) and food efficiency (7%) for the 10 week pre-mating period. The weights of the epididymides and kidneys were also significantly decreased in F_0 generation males. There were no corresponding effects in the F_1 parental generation except for decreased food efficiency (15%) in females for weeks 5-7 pre-mating. There were no detrimental effects of treatment with PHMB on reproduction in this study, but it is noted that there was a dose-related *decrease* in number of pup deaths days 1-5 post-partum for both generations.
The Parental Systemic Toxicity **NOEL** = 600 ppm (69.6 mg/kg/day [F_0 males]; 77.0 mg/kg/day [F_0 females]; 71.3 mg/kg/day [F_1 males]; 79.2 mg/kg/day [F_1 females]); The Parental Systemic Toxicity **LEL** = 2000 ppm (238.9 mg/kg/day [F_0 males]; 258.2 mg/kg/day [F_0 females]; 249.3 mg/kg/day [F_1 males]; 270.5 mg/kg/day [F_1 females]) based on decreased body weight and food efficiency in F_0 males and females, and decreased epididymis and kidney weights in F_0 males.

Reproductive / Systemic Toxicity **NOEL** = 2000 ppm; Reproductive / Systemic Toxicity **LEL** > 2000 ppm.

This study is classified as **acceptable** and **satisfies** the guideline requirement (OPPTS 870.3800; §83-4) for a 2-generation reproduction study in rats.

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Reviewed by: Timothy F. McMahon, Ph.D. _____ Date: 9/5/96
Pharmacologist, Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Stephen C. Dapson, Ph.D. Stephen C. Dapson Date: 9/5/96
Senior Pharmacologist, Section I, Toxicology Branch II (7509C)

Data Evaluation Record

Study type: Multigeneration Reproduction - Rat (§83-4)

EPA ID Numbers: MRID numbers: 43617401
Submission: S485943 DP Barcode: D214646
P.C. Code: 111801

Test material: Polyhexamethylene biguanide (PHMB)

Synonyms: Baquacil

Study number(s): RR0621/F0; RR0621/F1

Sponsor: Zeneca, Inc.

Testing Facility: Zeneca Central Toxicology Laboratory

Citation: Milburn, G.M. (1995). Polyhexamethylene Biguanide: Multigeneration Study in the Rat. Study performed by Zeneca Central Toxicology Laboratory. Study Nos. RR0621/F0; RR0621/F1. Study completed March 8, 1995. Unpublished. MRID # 43617401.

Executive Summary:

In a multigeneration reproduction study (MRID # 43617401), male and female Alpk:APfSD rats (26 males/dose; 26 females/dose), obtained from the Barriered Animal Breeding Unit at Zeneca Pharmaceuticals, Alderley Park, UK, received PHMB technical (20.2% a.i.) in the diet at nominal doses of 0, 200, 600, and 2000 ppm (23.0, 69.6, and 238.9 mg/kg/day for F_0 males; 25.3, 77.0, and 258.2 mg/kg/day for F_0 females; 23.9, 71.3, and 249.3 mg/kg/day for F_1 males; and 26.1, 79.2, 270.5 mg/kg/day for F_0 females). The rats in each generation received test diets continuously until termination. Systemic toxicity was observed at the 2000 ppm dose level in the F_0 generation as indicated by a decrease in group mean body weight (9-10%) and food efficiency (7%) for the 10 week pre-mating period. The weight of the epididymides and kidneys were also significantly decreased in F_0 generation males. There were no corresponding effects in the F_1 parental generation except for decreased food efficiency (15%) in females for weeks 5-7 pre-mating. There

were no detrimental effects of treatment with PHMB on reproduction in this study, but it is noted that there was a dose-related *decrease* in number of pup deaths days 1-5 post-partum for both generations.

The Parental Systemic Toxicity **NOEL** = 600 ppm (69.6 mg/kg/day [F_0 males]; 77.0 mg/kg/day [F_0 females]; 71.3 mg/kg/day [F_1 males]; 79.2 mg/kg/day [F_1 females]); The Parental Systemic Toxicity **LOEL** = 2000 ppm (238.9 mg/kg/day [F_0 males]; 258.2 mg/kg/day [F_0 females]; 249.3 mg/kg/day [F_1 males]; 270.5 mg/kg/day [F_1 females]) based on decreased body weight and food efficiency in F_0 males and females, and decreased epididymis and kidney weight in F_0 males.

Reproductive / Systemic Toxicity **NOEL** = 2000 ppm; Reproductive / Systemic Toxicity **LOEL** > 2000 ppm.

This study is classified as **acceptable** and **satisfies** the guideline requirement (OPPTS 870.3800; §83-4) for a 2-generation reproduction study in rats.

I. MATERIALS AND METHODS

A. Test Material: PHMB technical
purity: 20.2% in aqueous solution
reference no: Y00156/008
description: very faint yellow, mobile liquid

B. Vehicle: dietary administration

C. Test Animals: Species: Alpk:APfSD rats, male and female
Source: Barriered Animal Breeding Unit, Zeneca Pharmaceuticals.
Age: supplied as weanlings (22 days old)
Mean Weight: F_0 generation: males, 80.9-81.5 grams; females, 74.9-76.0 grams; F_1 generation: males, 73.2-83.6 grams; females, 68.7-74.9 grams.

D. Animal Husbandry

Sealed containers containing weanling rats from the Barriered Unit were transported to the SPF Barriered Unit at Zeneca Central Toxicology Laboratory and introduced into the Unit via a dunk tank. Access to the animal room was restricted for 10 days following delivery. Rats were housed in litters initially in multiple rat racks constructed of square section aluminum. Twenty cages each were suspended over stainless steel collecting trays lined with absorbent paper sheets. Temperature was maintained between 19-22 ^{o}C, and relative humidity at 45% minimum. The overall recorded temperature range was 17-28 ^{o}C, and the relative humidity was 23-98%. A twelve hour light/dark cycle was used, with 15 air changes per hour. Food was supplied in glass jars of approximately 300 gram capacity (CT1 diet), and filtered water was supplied via an automatic watering system. Water was also available from plastic bottles with ball-bearing nozzles for approximately one week after delivery of rats to ensure that the young rats obtained sufficient water until familiar with the automatic system. Food and water were available ad libitum. After being housed as litters, two males or two females per cage were assigned after experimental groups were determined. During mating, one male was housed with one female.

E. Dietary Preparation and Analysis:

CT1 diet, supplied by Special Diet Services Limited, Witham, Essex, UK, was used as the basis for the experimental diets and control diet, except during the pre-mix stage, when Laboratory Diet A was used as a pre-mix. This was done because, according to the report, "it gave a more homogeneous mix than CT1 with aqueous test substances. "

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Experimental diets were prepared in 60 kg batches . The appropriate amount of PHMB was mixed with 500g of Laboratory Diet A, mixed with a pestle in a mortar, to which was then added 2 x 250g of CT1 diet. The premixes were then added to 56-59 kg of diet and mixed thoroughly.

It is noted here that measurement of achieved concentration, homogeneity, and stability in diet was not performed. Homogeneity of the mixing procedure was determined on a 60 kg batch size, using an aqueous solution of a dyestuff. This procedure was performed at approximately 6 month intervals. The volumes of dye used were equivalent to the volumes of PHMB used to prepare the low and high dose level experimental diet. 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.

F. Procedures and Study Design

1) Mating:

Male and female rats in the same dose groups were housed in adjacent cages during the period prior to mating to avoid anestrus. During gestation and lactation, females were housed individually. During mating, one male was placed with a female from the adjacent cage. Vaginal smears were examined daily to determine mating, as shown by the presence of sperm. Gestation was presumed when abdominal enlargement and weight gain were seen (the day of sperm detection was designated day 1 of gestation). Females failing to show positive evidence of mating during the three week mating period were re-mated with another proven male from the same treatment group after a rest period of at least three days.

2) Mating schedule

After approximately 10 weeks on test diets, the F_0 rats were mated to produce the F_1 litter. Twenty-six male and 26 female rats per group were selected from the offspring (F_{1a}) to become F_1 parents. Offspring for the F_1 parental generation were selected on day 29 post-partum. Rats were selected from litters containing six to eighteen pups and excluded litters derived from remating. After selection, pre-mating and breeding was followed until an F_{2a} litter had been produced and weaned. Brother-sister mating was avoided in both generations.

3) Animal Assignment

F_0 rats were assigned to test groups as follows:

Test groups		Conc. (ppm)*	Animals per group ** Males	Females
1	Control	0	26	26
2	Low (LDT)	200	26	26
3	Mid	600	26	26
4	High	2000	26	26

*Diets were administered from the beginning of the study until the animals were sacrificed.
**The same number of animals were picked from the F0 litters as parents for the F1 generation.

G. Observation Schedule

1. Parental animals: Observations and the schedule for those observations is summarized from the report as follows:

Type of observation	Number of animals per sex per group	Frequency
Mortality and signs of toxicity	All	Once daily during pre-mating and growth periods.
Detailed clinical observations	All	Once daily during growth and breeding periods.
Body Weight	Male	At beginning of study and every 2 weeks through growth and mating periods
Body Weight	Female	At beginning of study; weekly during pre-mating; on days 1, 8, 15, and 22 of gestation; on days 1, 5, 11, 16, 22, and 29 of lactation.

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Food consumption	All	weekly during pre-mating period

2. Reproductive performance

The following indices were recorded :

$$\text{Female fertility index} = \frac{\text{Number of females delivering a litter}}{\text{Number of females co-housed with males}} \times 100$$

$$\text{Male fertility index} = \frac{\text{Number of males which sired a litter}}{\text{Number of males co-housed with females}} \times 100$$

In addition, the length of gestation and pre-coital interval were measured.

3. Litter observations: According to the report, the following litter observations were made:

	Time of observation (lactation day)					
Observation	Birth	Day 5	Day 11	Day 16	Day 22	Day 29
Number of live pups	x	x	x	x	x	x
Pup weight	x	x	x	x	x	x
External alterations	x	x	x	x		
Number of dead pups	x	x	x	x	x	x
Sex of each pup	x	x	x	x	x	x

In addition, the following indices were calculated for each litter:

$$\text{proportion of pups live born} = \frac{\text{number of pups born live}}{\text{no. of pups born live + no. of pups born dead}} \times 100$$

$$\text{proportion of pups surviving to day 22} = \frac{\text{number of pups alive on day 22}}{\text{number of pups alive on day 1}} \times 100$$

4) Necropsy

a. Parental Animals: All adult rats scheduled for termination as well as those requiring euthanasia for humane reasons were anesthetized with halothane and then killed by exsanguination. All rats dying or killed intercurrently received a full post-mortem examination as soon as possible and always within 24 hours of death. Females demonstrating prolonged gestation or parturition difficulties were killed. Females failing to show positive signs of mating and did not litter were killed at least three weeks after the last day of their mating period. All remaining females were killed as soon as possible after weaning of their offspring.

Parental animals were subjected to post mortem examination as follows:

Animals examined	Macroscopic	Microscopic
Found dead	x	x
Unscheduled sacrifice	x	x
Scheduled sacrifice	x	x

The kidney, liver, testes, and epididymides were weighed in all adults at termination. Kidneys were weighed together. The following tissues were submitted: kidney, liver, cervix, epididymis, mammary gland (females only), ovary, pituitary gland, prostate gland, seminal vesicles including coagulating gland, testis, uterus, vagina, and grossly abnormal tissues. The uterus was examined in addition for implantation sites and their presence or absence recorded.

Five micron sections of all processed tissues from the **control** and **2000ppm** dose groups and from suspected infertile adults in the remaining groups were cut and stained with hematoxylin and eosin. All sections were examined by light microscopy.

b. Offspring:

Five male and 5 female F_{1a} pups per dose group and 10 male and 10 female F_{2a} pups per dose group were scheduled to receive a full post-mortem examination. These pups were randomly selected with the proviso that not more than one pup of each sex was chosen from any one litter. The remaining pups showing any clinical abnormality were given a gross post-mortem, and, where possible, two of any remaining clinically normal pups of each sex from each litter were given a gross post-mortem examination. Pups over 18 days of age dying or killed intercurrently were also given a full post-mortem examination.

The kidney, liver, testis, and epididymis were weighed in all pups receiving a full post-mortem examination. These same organs were also weighed from 5 F_{1a} pups per sex per group which received a gross post-mortem examination. Tissues submitted for a full post-mortem examination in pups were the same as those for adults except that the mammary gland was not submitted. Only macroscopically abnormal tissues were submitted from pups which received a gross post-mortem examination.

The kidney and liver from all F_{1a} pups receiving a full post-mortem at termination, the kidney and liver in all F_{2a} pups in the control and 2000 ppm dose group that received a full necropsy at termination, and the eyes from 2 male F_{2a} pups receiving 2000 ppm PHMB (in which a gross abnormality was detected in the left eye) were processed. Five micron sections were cut and stained with hematoxylin and eosin and examined by light microscopy.

H. Data Analysis

Analysis of variance was used for calculation of the statistical significance of the following parameters:

- weekly food consumption, parental organ weights, initial pregnancy and lactation bodyweights, pregnancy and lactation food consumption, litter size, mean gestation length, mean pre-coital interval, initial pup weight, total litter weight.

Analysis of covariance was used for calculation of the statistical significance of the following parameters:

- Pregnancy and lactation bodyweights after day 1, mean pup weights after day 1.

Proportion of fertile animals, proportion of whole litter losses, proportion of litters with gestation length 22 and > 22 days and the proportion of litters with pre-coital interval of length 1, 2, 3, 4, and >4 days in each treatment group were considered by Fisher's Exact Test.

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II. REPORTED RESULTS

A. Parental Animals

1. Mortality and clinical signs: Clinical signs of toxicity, including mortality, were presented for the F_0 and F_1 parental generation in Tables 2 and 3, pages 75-85 of the report. According to the data presented, there were no significant treatment-related increases in the incidence of clinical signs or in mortality in either the F_0 or F_1 parental rats.

2. Body weight and food consumption:

Reported body weight (mean ± S.D.) and selected food consumption (mean ±S.D.) results are summarized as follows:

F_0 Generation Males - Pre-mating

Observation and study week	Dose group 0 ppm	200ppm	600ppm	2000ppm
N =	26	26	26	26
Mean body weight (g)				
week 1	80.9±5.3	81.6±6.9	81.5±4.5	81.3±6.0
week 8	392.5±23.8	384.3±29.8	381.0±17.8	362.8±23.3
Mean weight gain (g)				
weeks 1 - 8	311.6	302.7	299.5	281.5
Mean food consumption (grams)				
week 1	20.9±1.2	20.9±1.9	20.6±1.1	21.0±1.4
week 2	27.3±1.5	26.7±1.7	27.0±1.6	26.7±1.4
week 3	31.0±1.0	30.3±1.5	30.2±1.1	29.7±1.4**

F_0 Generation Females - Pre-mating

Observation and study week	Dose group 0ppm	200ppm	600ppm	2000 ppm
N =	25	26	26	26
Mean body weight (g)				
week 1	75.1±4.9	76.0±4.4	74.9±5.7	75.1±5.6
week8	231.3±15.7	232.7±16.6	226.5±18.3	218.0±17.3
Mean weight gain (g)				
weeks 1 - 8	156.2	156.7	151.6	142.9
Mean food consumption (grams)				
week 1	18.8±0.6	19.0±0.9	19.6±2.0	19.4±2.4
week 4	23.4±2.0	23.5±1.2	23.4±2.2	22.4±2.2
week 5	23.1±1.5	23.6±1.0	22.9±2.2	22.2±1.5
week 6	22.8±1.4	23.4±1.3	22.9±1.4	22.1±1.3
week 7	23.9±1.7	24.4±1.0	23.9±1.9	23.0±1.2
week 8	24.4±1.6	24.5±1.5	24.6±1.6	23.3±1.4

*Statistically significantly different from control by ANOVA, $p<0.05$.

F_1 Generation Males - Pre-mating

Observation and study week	Dose group 0ppm	200ppm	600ppm	2000ppm
N =	25	26	26	26
Mean body weight (g)				
week 1	83.6±10.0	73.2±9.3**	79.7±9.4	76.4±8.1**
week 10	420.4±40.2	404.1±34.6	417.9±28.2	396.0±34.4
Mean weight gain (g)				
weeks 1 - 10	336.8	330.9	338.2	319.6

Mean food consumption (grams)				
week 1	20.6±0.9	19.0±1.1**	20.2±1.4	19.6±1.8
week 6	33.7±2.0	32.6±1.5	33.5±1.6	34.1±3.2
week 10	34.6±2.4	32.0±2.1*	33.0±1.9	33.8±3.9

F_1 Generation Females - Pre-mating

	Dose group			
Observation and study week	0 ppm	200ppm	600ppm	2000ppm
N =	25	26	26	26
Mean body weight (grams)				
week 1	74.9±7.7	68.7±7.8**	72.6±7.7	70.1±5.8*
week 10	249.6±18.8	240.4±19.8	246.4±14.7	236.5±14.9
Mean weight gain (g)				
week 0 - 10	174.7	171.7	173.8	166.4
Mean food consumption (grams)				
week 1	17.9±1.0	16.9±1.1*	17.9±1.4	17.4±0.6
week 4	24.0±1.8	23.4±2.5	24.3±2.4	24.0±3.3
week 8	23.5±1.2	22.9±1.9	23.7±1.2	24.4±3.1
week 10	23.8±1.0	23.7±2.3	23.5±1.1	24.9±2.9

* Statistically significantly different from control by ANOVA, $p<0.05$.

In the F_0 generation, male and female rats in the high dose (2000ppm) dose group showed decreased group mean body weight gain for weeks 1-8, but the decrease was marginal (9-10%). Similar decreases were not observed at lower dose levels, and food consumption was not significantly affected at any dose level. In the F_1 parental generation, male and female rats at the high dose level showed only minor decreases in body weight gain for the pre-mating period (5% decrease for weeks 1-10). Again, food consumption was not significantly affected at any dose level in the F_1 parental generation. The report noted for F_0 males (page 103) a decrease in food utilization (efficiency) at the high dose for weeks 1-4 (decrease of 5%), weeks 5-7 (decrease of 12%), and weeks 1-10 overall (decrease of 7%) vs control. A similar decrease in food utilization was reported for F_1 male rats at the high dose for weeks 1-4 and weeks 1-10. The only significant change in food utilization noted for female rats was for the F_1 generation, where food utilization was decreased 15% at the high dose during weeks 5-7.

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Compound Intake

Compound intake during the pre-mating period and during pregnancy and lactation for both parental generations of rats was summarized on pages 192-195 of the report. For the F_0 generation, the mean compound intake for the 10-week pre-mating period was calculated as 23.0, 69.6, and 238.9 mg/kg/day for F_0 males; 25.3, 77.0, and 258.2 mg/kg/day for F_0 females; 23.9, 71.3, and 249.3 mg/kg/day for F_1 males; and 26.1, 79.2, 270.5 mg/kg/day for F_0 females. For pregnant females, compound intake for the F_0 females was reported as 18.6, 56.9, and 187.7 mg/kg/day, and compound intake for the F_1 females was reported as 19.3, 57.0, and 201.5 mg/kg/day. During lactation, compound intake for F_0 females was calculated as 50.7, 150.8, 537.3 mg/kg/day; for F_1 females, 44.6, 133.6, and 486.3 mg/kg/day.

Selected group mean body weight values for pregnant or nursing dams are summarized as follows:

F_0 Generation - Female

Observation and study week	Dose group 0 ppm	200ppm	600ppm	2000ppm
N =	24	23	24	25
Mean body weight (grams)				
Day 1 of gestation	260.5±16.3	264.9±16.2	255.8±15.7	246.4±17.2**
Day 22 of gestation	385.2±20.1	391.2±26.4	378.5±22.8	363.1±22.6
Mean body weight (g)				
Day 1 of lactation	292.3±19.2	296.5±17.5	288.7±19.5	281.4±24.5*
Day 22 of lactation	336.6±16.9	342.5±16.2	341.2±19.3	339.9±17.7
Mean body weight gain (g)				
Days 1-22 of gestation	124.7	126.3	122.7	116.7
Day 1-22 of lactation	44.3	46	52.5	58.5

F_1 Generation-Female

Observation and study week	Dose group 0ppm	200ppm	600ppm	2000ppm
N =	24	25	26	26
Mean body weight (g)				
Day 1 of gestation	267.9±22.9	257.0±20.6	266.6±20.7	253.3±17.0 *
Day 22 of gestation	400.0±30.8	392.1±26.2	399.7±26.0	390.4±23.6
Day 1 of lactation	306.7±29.7	297.9±22.4	307.8±22.6	287.7±19.5*
Day 22 of lactation	349.5±21.2	350.9±21.9	355.7±22.6	350.0±18.9
Mean body weight gain (g)				
Days 1-22 of gestation	132.1	135	133.1	137.1
Day 1-22 of lactation	42.8	53	47.9	62.3

*Statistically significantly different from control by ANOVA, $p<0.05$.

For the F_0 generation, the only effect noted on body weight was on day 1 of gestation, when body weight at the high dose was decreased by 6% vs control, and on day 1 of lactation, when body weight at the high dose was decreased by 4% vs control. Overall weight gain for the F_0 females in the high dose group during gestation was decreased by 7% vs control.

In the F_1 generation, the only effect noted during gestation and lactation on body weight was a 6% decrease in body weight at the high dose on day 1 of gestation and lactation.

4. Reproductive performance

Results for the parental animals are summarized from the report (Tables 11-15, pages 109-113 of the report):

F_0 Generation

Observation and study week	Dose group 0 ppm	200ppm	600ppm	2000ppm
Median precoital interval (days)[a]	2.79±3.21	3.43±3.46	2.34±1.17	1.84±0.97
Males				
Mated[b]	26	26	26	26
Fertile[c]	21	21	22	22
Infertile	5	5	4	4
Intercurrent deaths	0	0	0	0
Females				
Number mated[c]	26	26	26	26
Number fertile	25	23	24	25
Infertile	0	3	2	1
Intercurrent deaths	1	1	1	0
Mean gestation interval (days)	22.2±0.4	22.2±0.4	22.2±0.4	22.2±0.4
Number of litters[d]	24	23	24	25
Total litter losses	0	1	1	0
Mean litter size (Day 1)	12.0	12.2	11.6	11.5
Mean litter size (Day 5)	10.4	11.4	10.9	11.0
Mean litter size (Day 22)	10.3	11.2	10.8	10.9

F_0 Generation, cont.

Observation and study week		Dose group 0 ppm	200ppm	600ppm	2000ppm
Number of live pups (Day 0)[e]		287	270	267	287
Number of live pups (Day 22)		246	246	248	273
Pup deaths (Days 1-5)[e]		37	20	17	13
Pup deaths (Days 5-22)[e]		4	4	2	1
Mean pup weight (g) (Day 1)	Male	6.3	6.3	6.3	6.1
	Female	5.9	6.0	5.8	5.8
Mean pup weight (g) (Day5)	Male	9.5	9.1	9.2	9.1
	Female	9.0	8.7	8.7	8.8
Mean pup weight (g) (Day 11)	Male	19.2	18.2	18.5	18.0
	Female	18.3	17.5	17.7	17.5
Mean pup weight (g) (Day 22)	Male	43.8	40.2	41.3	40.0
	Female	41.4	38.8	39.6	38.7

*Statistically significantly different from control, $p<0.05$.

a- obtained from page 109 of the report
b- obtained from page 342-343 of the report.
c- obtained from page 342-343 of the report.
d- obtained from page 113 of the report.
e- obtained from page 342-349 of the report.

F_1 Generation

Observation and study week	Dose group 0 ppm	200ppm	600ppm	2000ppm
Median precoital interval (days)[a]	2.28±0.98	2.92±2.88	2.63±2.03	2.30±1.07
Males				
Mated[b]	26	26	26	26
Fertile[b]	22	26	22	24
Infertile[b]	4	0	4	2
Intercurrent deaths	0	0	0	0
Females				
Number mated[b]	26	26	26	26
Number fertile[b]	26	26	26	26
Infertile	0	0	0	0
Intercurrent deaths	0	0	0	0
Mean gestation interval (days)	22.5±0.5	22.3±0.5	22.3±0.5	22.3±0.5
Number of litters[c]	25	25	26	26
Total litter losses[c]	6	6	2	3
Mean litter size (Day 1)	11.2	11.8	12.0	13.3*
Mean litter size (Day 5)	8.6	10.2	10.0	11.0*
Mean litter size (Day 16)	8.4	9.8	9.8	10.8*
Mean litter size (Day 22)	8.4	9.8	9.8	10.8*
Number of live pups (Day 1)	257	289	315	345
Number of live pups (Day 22)	160	186	236	248
Pup deaths (Days 1-5)	91	85	64	67
Pup deaths (Days 5-22)	2	8	3	5

F_1 Generation, cont.

Observation and study week		Dose group 0 ppm	200ppm	600ppm	2000ppm
Mean pup weight (g) (Day 1)	Male	6.1	5.9	6.1	5.9
	Female	5.8	5.5	5.6	5.5
Mean pup weight (g) (Day 5)	Male	8.8	8.1	8.2	8.2
	Female	8.3	7.7	8.0	7.8
Mean pup weight (g) (Day 16)	Male	30.1	27.8	27.9	27.5
	Female	29.4	26.7	27.0	26.4
Mean pup weight (g) (Day 22)	Male	47.4	42.0	41.9	40.4
	Female	45.3	40.2	40.4	38.7

*Statistically significantly different from control, $p<0.05$.

a- obtained from page 119 of the report
b- obtained from pages 1191-1198 of the report.
c- obtained from page 112 of the report.

For the parameters reported above, there were no detrimental effects of treatment with PHMB at any of the dose levels employed. There was, however, a significant increase in mean litter size for the F_1 generation at the 2000 ppm dose. The number of pup deaths from days 1-5 also appeared to be decreased in a dose-related fashion in both generations.

5. Necropsy results

a. Organ and Final Body Weights: A summary of organ weights for the F_0 generation parents was presented on pages 127-130 of the report, while a summary of organ weights for the F_1 generation parents was presented on pages 131-134 of the report.

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Terminal Body Weights and Organ Weights in F_0 and F_1 Parental Rats

F_0 Generation

Observation	Dose group 0 ppm	200ppm	600ppm	2000ppm
Males				
Terminal body weight (g)	528.3±42.7	515.9±48.8	509.6±34.1	488.9±34.9
Epididymide weight (g)	1.56±0.17	1.48±0.16	1.44±0.12**	1.43±0.14**
Kidney weight (g)	3.55±0.31	3.45±0.38	3.38±0.30*	3.44±0.36
Liver weight (g)	20.0±2.5	19.7±2.4	19.1±2.3	19.8±1.9
Females				
Terminal body weight (g)	310.3±15.8	308.4±17.0	306.3±18.8	301.4±22.8
Kidney weight (g)	2.41±0.21	2.42±0.22	2.36±0.19	2.45±0.24
Liver weight (g)	14.9±2.4	14.2±1.9	14.0±1.8	14.8±2.6

F_1 Generation

Observation	Dose group 0 ppm	200ppm	600ppm	2000ppm
Males				
Terminal body weight (g)	539.1±50.8	508.0±47.0	521.8±32.0	498.4±43.7
Epididymide weight (g)	1.52±0.18	1.50±0.29	1.51±0.18	1.49±0.14
Kidney weight (g)	3.53±0.35	3.43±±0.45	3.45±0.33	3.45±0.30
Liver weight (g)	20.7±2.2	18.9±2.1**	19.6±1.8	20.5±2.4
Females				
Terminal body weight (g)	313.3±19.1	309.7±23.0	315.5±27.4	304.5±20.7
Kidney weight (g)	2.41±0.25	2.36±0.25	2.42±0.26	2.50±0.26
Liver weight (g)	13.4±1.8	13.3±1.9	14.1±3.2	14.0±2.0

*statistically different from control, $p < 0.05$.

The above data on terminal body weight show that in male rats, terminal body weight at the 2000 ppm dose level was decreased by 7-8% for both the F_0 and F_1 generations. No effect on terminal body weight was observed in female rats. The absolute weight of the epididymides was decreased by approximately 8% in male rats at the 600 and 2000 ppm dose levels. Other organ weights did not appear to be significantly affected in parental rats in this study at any dose level tested.

b. Pathology

i. Macroscopic examination

Gross examination data for F_0 rats was presented in Tables 24 and 25, pages 143-149 of the report, and gross examination data for F_1 rats was presented in Tables 26 and 27, pages 150-154. There were no reported macroscopic abnormalities in male or female F_0 adults. In the F_1 adults, an increased incidence of pelvic dilatation of the kidney was reported for female rats at the 2000ppm dose level (8/26 rats examined vs. 3/25 rats examined in control). There were no other reported macroscopic abnormalities reported for F_1 adults.

ii. Microscopic examination

Results of microscopic examination of the F_0 and F_1 parental rats was shown in Tables 28 and 29 of the report, pages 155-162. There were no reported microscopic abnormalities in the F_0 parental generation. In the F_1 parental generation, an increase in the incidence of female rats with slight unilateral hydronephrosis was observed at the 2000ppm dose level (6/26 examined vs. 1/26 examined in control). Minimal intratubular microlithiasis (presence of minute concretions or gravel) was also observed in increased incidence in female rats at the 2000ppm dose level (8/26 examined vs. 2/26 examined in control).

C. Offspring

1. Viability and clinical signs:

Viability results from pups during lactation are summarized from the report as follows:

F_{1a} Litter (page 116 of the report)

Observation	Dose Group (ppm) 0	200	600	2000
Mean percentage surviving in each litter (day 22)	87.4	91.2	93.8	95.6*
No. litters with all pups surviving to Day 21 / total no. litters	11/24 (46%)	11/22 (50%)	14/23 (61%)	15/25 (60%)

F_{2a} Litter (page 116 of the report)

Mean percentage surviving in each litter (day 22)	76.3	81.6	82.6	82.7**
No. litters with all pups surviving to Day 22 / total no. litters	6/18 (33%)	5/19 (26%)	6/24 (25%)	7/23 (30%)

*Statistically different from control, $p < 0.05$. ** statistically different from control, $p < 0.01$.

The above data show that there does not appear to be a detrimental effect of PHMB treatment on pup survival. However, it is noted that the number of litters with all pups surviving to day 22 out of all litters resulted in low percentages for both the F_{1a} and the F_{2a} litter, especially the F_{2a} litter. It is also noted from previous data presented that the number of pup deaths from days 1-5 post-partum was high in relation to the number of pup deaths from days 5-22 for both litters. These could be the result of what was described in the report (page 38) as "an environmental disturbance due to building work in an adjacent animal block." The report went on to say that "litter losses did not affect the ability of the study to detect effects of PHMB on reproduction as a satisfactory number (between 19 and 24) of F_{2a} litters per group survived to weaning and provided information on the growth of offspring receiving PHMB." It is likely that there was maternal stress involved as a result of the "environmental disturbance" and that nursing of offspring could have been compromised, resulting in the low survival percentages.

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Changes in mean number of pups/litter as well as changes in group mean body weight for the F_1 and F_2 litters were summarized above on pages 14-16 of this review.

3.Necropsy results

a. Organ weights:

Organ weight data for the F_{1a} and F_{2a} pups was presented in Tables 23.1 - 23.2, pages 135-142 of the report. For the F_{1a} pups, the weight of the epididymides in male pups was decreased 9% at the 2000ppm dose (0.087 grams vs. 0.095 grams in control). Kidney weight was also decreased in high dose male pups by 12% (0.97 grams vs. 1.09 grams in control. $p < 0.05$). There were no reported effects on organ weight in female F_{1a} pups.

In the F_{2a} litter, the weight of the epididymides in male pups was decreased at all dose levels (decreases of 32%, 40%, and 21% in the 200, 600, and 2000 ppm dose groups, respectively) as was the weight of the testes at the 600 and 2000ppm dose groups (decreases of 23% and 17%, respectively). Kidney weight in female rats was decreased at the 2000 ppm dose (0.82 grams) in relation to control (0.96 grams), a decrease of 15%. Liver weight in male pups was significantly decreased at the 600 and 2000ppm dose levels (decreases of 17% and 16%, respectively), as was liver weight in female pups from the 2000 ppm dose group (25% decrease). However, liver weight was also significantly decreased in female pups at the low dose (decrease of 13%), so the significance of the high dose decrease is not clear.

b. Pathology

i. Macroscopic examination:

Data on macroscopic examination of the F_1 and F_2 litters were reported (pages 92 and 124 of the report). These data showed no treatment-related effects in either generation of litters.

ii. Microscopic Observations

Microscopic findings for F_{1a} and F_{2a} pups were summarized in Tables 32 and 33, pages 171-174 of the report. A slight increase in the incidence of unilateral hydronephrosis (graded as slight)was observed in female pups of the F_{1a} generation at the 2000 ppm dose level (2/5 pups

examined vs. 0/5 examined in control). Tubular basophilia (graded as minimal) was observed in 2/5 high dose female pups compared to 1/5 control female pups. In the F_{2a} litter, one high dose male was reported with marked keratitis and iritis with anterior synechia formation and hemorrhage into the anterior chamber. The report considered this to be the result of infection following trauma to the eye and not to treatment with PHMB. The incidence of transitional epithelial hyperplasia (7/10 high dose male pups vs 3/10 controls), tubular basophilia (4/10 high dose male pups vs 2/10 control), and tubular dilatation (2/10 high dose male pups vs 1/10 control) was also increased slightly. The report considered these spontaneous common changes and stated that the incidence in treated males was comparable to that observed in control female pups.

III. DISCUSSION

A. Investigators' conclusions

According to the study report, dietary exposure of parental rats to 2000ppm PHMB resulted in decreased body weight in male and female rats. This decrease was accompanied by reduced food efficiency. This effect was considered to be most evident in the F_0 generation. There was no effect of treatment with PHMB on any of the reproductive parameters examined. An unexpected finding was the high number of litter deaths in the F_{2a} litter. However, there were more litters lost in the control group than at the 600 or 2000 ppm dose level. The report attributed the loss to an environmental disturbance due to building work in an adjacent animal block. The report concluded that there was no treatment related pathology in either adult rats or offspring. There were slight increases in the weight of the kidney and liver of F_0 male rats and F_1 male and female rats receiving 2000 ppm PHMB, but in the absence of pathological changes, the organ weight changes were considered unrelated to treatment. The decreased weights of the epididymides in F_0 male rats were also considered unrelated to treatment as there were no microscopic abnormalities noted, and there was no similar effect in F_1 males. The organ weight changes in the F_{2a} pups were unrelated to treatment as there was no evidence of a dose response, and there was no supporting histopathological evidence. The report concluded that the No Observed Adverse Effect Level for PHMB in adult rats was 600 ppm, and the No Observed Adverse Effect Level in the offspring was 2000 ppm.

B. Reviewer's discussion

In the present study, the reproductive toxicity of PHMB technical was assessed in male and female Sprague-Dawley rats. Male and female rats (26 males/dose; 26 females/dose), obtained from the Specific Pathogen Free colony maintained at the Barriered Animal Breeding Unit at Zeneca Pharmaceuticals, received PHMB technical (20.2% a.i.) in the diet at nominal doses of **0, 200, 600, and 2000 ppm** (nominal mg/kg/day doses of 23.0, 69.6, and 238.9 mg/kg/day for F_0 males; 25.3, 77.0, and 258.2 mg/kg/day for F_0 females; 23.9, 71.3, and 249.3 mg/kg/day for F_1 males; and 26.1, 79.2, 270.5 mg/kg/day for F_0 females). The issue of dietary concentration of PHMB has been addressed previously, as it is known that PHMB binds to diet and being a polymer, cannot be quantitatively measured. The Agency and the registrant have previously agreed that assessment of dietary concentration and homogeneity using a dyestuff in place of PHMB would be acceptable.

There were limited effects in the present study for assessing the reproductive toxicity of PHMB. The body weight decreases in the F_0 generation were marginal (9-10%), although food efficiency was also decreased to a similar degree for the 10-week pre-mating period (7%). In the F_1 parental generation, the only effect noted was a decrease in food efficiency of 15% for weeks 5-7 pre-mating. There was a lack of any significant effect on reproduction at any dose level tested in the present study. It is noted that there was an unusually high number of pup deaths for days 1-5 post-partum in both the F_0 and F_1 generations. The number of litters with all pups surviving to day 22 was also low. These effects could have been the result of maternal stress due to the environmental disturbance occurring in an adjacent animal facility. The report mentioned decreased epididymis weight observed in the F_0 male rats at the 2000 ppm dose (8% decrease vs. control), but did not mention that decreased weight of the epididymides were also observed in the F_{1a} pups at 2000 ppm (9%) as well as the F_{2a} pups at all doses (32, 40, and 21% at the 200, 600, and 2000 ppm dose levels, respectively). Testes weight in the F_{2a} pups was also decreased by 23% and 17% at the 600 and 2000 ppm dose levels, respectively. Although these decreases are not consistently dose-related or observed consistently between generations, an effect of PHMB on the testes has been reported from administration of 4500 ppm PHMB to male dogs for one year (MRID # 43623501). In that study, testis weight was decreased by 29% and 32% for the left and right testis, respectively at the high dose level of 4500 ppm. Thus, the testis or associated structures might be involved as a target organ of PHMB toxicity.

The results of this study indicate slight toxicity at the 2000 ppm dose level, indicated by decreased body weight and food efficiency in the F_0 parental generation, and by possible effects on the testes

and epididymides of F_{1a} and F_{2a} pups. There were no significant effects on reproduction in this study, although it is noted that there was a dose-related *decrease* in the number of pup deaths from days 1-5 post-partum.
The Parental Systemic Toxicity **NOEL** = 600 ppm (69.6 mg/kg/day [F_0 males]; 77.0 mg/kg/day [F_0 females]; 71.3 mg/kg/day [F_1 males]; 79.2 mg/kg/day [F_1 females]); The Parental Systemic Toxicity **LOEL** = 2000 ppm (238.9 mg/kg/day [F_0 males]; 258.2 mg/kg/day [F_0 females]; 249.3 mg/kg/day [F_1 males]; 270.5 mg/kg/day [F_1 females]) based on decreased body weight and food efficiency in F_0 males and females, and decreased epididymis and kidney weight in F_0 males.

Reproductive / Systemic Toxicity **NOEL** = 2000 ppm; Reproductive / Systemic Toxicity **LOEL** > 2000 ppm.

IV. CLASSIFICATION: acceptable

This study is **acceptable** and satisfies the data requirements for a 2-generation reproductive toxicity study (OPPTS 870.3800; §83-4) in rats.