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

NOV 29 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Polyhexamethylene Biguanide (PHMB; Baquacil): Review of Toxicology Data Submitted by the Registrant.

Shaugnessey: 111801
Submission: S447287
MRID No: 428659-01
DP Barcode: D194755

FROM: Timothy F. McMahon, Ph.D., Toxicologist 11/23/93
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and

Marcia Van Gemert, Ph.D., Branch Chief 11/24/93
Toxicology Branch II
Health Effects Division (7509C)

Registrant: Zeneca, Inc.

Action Requested: Review of a developmental toxicity study submitted in support of reregistration of baquacil.

1 of 16

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Data Summary:

A study entitled, "Polyhexamethylene Biguanide: Developmental Toxicity Study in the Rabbit" was submitted by Zeneca, Inc. for review. This study was conducted at Zeneca Central Toxicology Laboratory and was completed in July of 1993.

In this study, administration of PHMB technical to pregnant female New Zealand White rabbits resulted in maternal toxicity at 40 mg/kg/day in the form of increased mortality and clinical toxicity. There was evidence of possible developmental toxicity of PHMB at 40 mg/kg/day, in the form of reduced number of litters and skeletal abnormalities (non-ossified 5th sternebrae, fused 3rd, 4th, and 5th sternebrae).

Maternal NOEL= 20 mg/kg/day
Maternal LOEL= 40 mg/kg/day (increased mortality; reduced food consumption; clinical toxicity)

Developmental toxicity NOEL = 20 mg/kg/day
Developmental toxicity LEL = 40 mg/kg/day (reduced no. of litters, skeletal abnormalities)

CLASSIFICATION: Core minimum

This study satisfies the guideline requirement (§83-3) for a developmental toxicity study in rabbits.

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Reviewed by: Timothy F. McMahon, Ph.D. 11/23/93
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Stephen C. Dapson, Ph.D. Stephen C. Dapson 11/23/93
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Data Evaluation Report

Study type: Developmental Toxicity- Teratology
Species: rabbit
Guideline: 83-3

EPA ID Numbers: MRID number: 428659-01
ToxChem No: 111801
Submission: S447287
DP Barcode: D194755

Test material: Polyhexamethylene biguanide (PHMB)

Synonyms: Baquacil

Study number(s): RBO 614

Testing Facility: Zeneca Central Toxicology Laboratory
Alderly Park, Cheshire, UK

Sponsor: Zeneca Inc., Wilmington, Delaware

Title of report: Polyhexamethylene Biguanide: Developmental Toxicity Study in the Rabbit

Author(s): A. Brammer

Study Completed: July 14, 1993

Conclusions: Administration of PHMB technical to pregnant female New Zealand White rabbits resulted in maternal toxicity at 40 mg/kg/day in the form of increased mortality and clinical toxicity. There was evidence of possible developmental toxicity of PHMB at 40 mg/kg/day, in the form of reduced number of litters and skeletal abnormalities (non-ossified 5th sternebrae, fused 3rd, 4th, and 5th sternebrae).

Maternal NOEL= 20 mg/kg/day
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CLASSIFICATION Core minimum

This study satisfies the guideline requirement (§83-3) for a developmental toxicity study in rabbits.

I. MATERIALS and METHODS

A. Test Material: polyhexamethylene biguanide
composition: 20.2%% a.i.
description: faint yellow liquid
CTL reference # Y00156/008
Note: the reference # on page 62 is stated as Y0156/008/001.

B. Vehicle: deionized water (CTL reference # Y04517/015)

C. Compound Stability and Homogeneity: Chemical stability of PHMB in deionized water had been determined previously by re-analysis of formulations at levels of 0.02, 7.0, and 80 mg/ml after a minimum interval of 21 days. The method of analysis was stated in Appendix B, page 64 of the report, and the results (page 31 of the report) showed mean concentrations of PHMB between 91-105% of nominal after storage for up to 124 days at room temperature.

D. Test Animals: Species: New Zealand White Rabbit, female.
Source: Conventional Animal Breeding Unit, Cheshire, UK
Age: not stated

Weights of control and treated groups on **day 1**, with the range and mean, are as follows:

dose group	range	mean
control	3225-4050	3601.6± 275.7
low dose	3141-4337	3490.7±310.1
mid dose	3241-4049	3483.7±236.5
high dose	3220-4307	3587.3±366.7

E. Animal Husbandry:

A total of 80 female rabbits were used in this study. Rabbits were mated with unrelated males of the same strain at the suppliers', and provided to the laboratory as time-mated females. Day of mating was designated as day 1 of gestation. The animals were delivered to the laboratory over a 2 week period. Rabbits were housed individually in mobile rabbit units in a temperature (15-19 ^{o}C) and humidity (40-70%) controlled room with a 12 hour light/dark cycle). Each cage was suspended over a collecting tray which was cleaned by a flushing system, and removable food hoppers were connected to the front of each cage. Rabbits were individually identified by ear tag. Food (BeeKay Rabbit Maintenance Diet) and water were available ad libitum. Water was also provided in a bowl within each rabbit cage from the time of arrival of each rabbit until an indication of satisfactory food intake was observed. A weighted quantity of food was provided to each rabbit on days 1, 4, 8, 11, 14, 17, 20, 23, and 26 of the study, and the amount consumed was calculated from the amount left on these same days.

F. Experimental Design and Dosing:

PHMB Technical was administered in deionized water by gavage to female rabbits on gestation days 8 through 20 inclusive in order to assess developmental toxicity of this chemical. Control animals received the appropriate volume of deionized water. Dose volume was adjusted based on individual body weights.

Doses and numbers of rabbits tested at each dose level are as follows:

0 mg/kg/day:	20 rabbits	(animal numbers 1-20)
10 mg/kg/day:	20 rabbits	(animal numbers 21-40)
20 mg/kg/day:	20 rabbits	(animal numbers 41-60)
40 mg/kg/day:	20 rabbits	(animal numbers 61-80)

G. Mating

As stated, mating was performed at the animal supplier. Details of mating were not provided, but it appears that insemination was by natural means.

H. Statistical Analysis:

A copy of the statistical tests used in these studies and the purposes for which they were employed is appended to this report.

I. Compliance:

A signed statement of Compliance with Good Laboratory Practice Standards was provided.

A signed statement of Data Confidentiality Claims was provided.

A signed statement of Quality Assurance was provided.

A signed statement of Flagging of Studies for Potential Adverse Effects was provided. This study neither meets nor exceeds the criteria as set forth in 40 CFR 158.35.

II. OBSERVATIONS and RESULTS:

A. Maternal Toxicity

1. Mortality and Clinical Signs

All animals were observed at least twice daily for signs of abnormal clinical condition or mortality. Animals requiring euthanasia were killed by intravenous pentobarbitone overdose. These animals, together with those found dead were given a macroscopic examination post mortem, and pregnancy status determined.

There was one intercurrent death in the control group, as a result of an infection arising from a leg injury sustained during delivery on day 1.

All other deaths occurred at the 40 mg/kg/day dose group, and involved a total of 6 rabbits. One rabbit (no.61) was killed in extremis on day 22 due to inappetance and weight loss present from day 11. Animal no. 75 was found dead on day 20, and post-mortem examination showed blood present in the thoracic cavity, suggesting a possible mis-dosing. The remaining 4 rabbits died as a result of abortion between days 26-30 of the study. Inappetance and weight loss were present in these 4 rabbits during the dosing and post-dosing periods.

Clinical observations were summarized in Table 5, pages 35-38 of the report. The incidence of several clinical signs appeared increased in the 40 mg/kg/day dose group, and included coldness (6/20 vs 0/20 in control), few feces (16/20 vs 7/20 in controls), no feces (6/20 vs 0/20 in controls), thin appearance (6/20 vs 0/20 in controls), and subdued behavior (3/20 vs 1/20 in controls).

2. Body Weight:

Body weight of each rabbit was recorded on arrival and again on days 4, 8 through 20 inclusive, and on days 23, 26, and 30 of gestation. Group mean body weights and individual body weight data were provided. Group mean body weight gain is shown in the following table (**Table 1**):

TABLE 1

Group Mean Body Weight Gains (g) in PHMB Technical-Treated Rabbits[a]

Study Interval (days)	Dose groups (mg/kg/day)			
	0	10	20	40
1-8	96	109	114	99
8-20	251	121	215	174
20-30	185	243	258	265
1-30	532	571	587	538

[a]Data calculated from Table 6, pages 39-40 of the report

There were no apparent treatment-related changes in body weight gain in rabbits dosed with PHMB at any of the dose levels tested. Although apparent decreases in body weight gain were observed in those rabbits at the 40 mg/kg/day dose level during days 8-20 of the study, a similar decrease was also observed at the 10 mg/kg/day dose level. Overall weight gain for days 1-30 of the study was not significantly affected in any group of rabbits.

Statistically significant decreases in absolute body weight were identified in Table 6 of the report at the 40 mg/kg/day dose level, from day 10 through 15. However, these decreases amounted to approximately a 2% decrease in absolute weight compared to control body weight.

4. Food consumption

As mentioned above, food consumption was measured for each animal by subtracting the amount left from the amount provided on study days 4, 8, 11, 14, 17, 20, 23, 26, and 30. Data on group mean food consumption and individual food consumption were provided by the registrant. Food consumption data are summarized in the following Table (**Table 2**).

TABLE 2

Food Consumption (grams/day) in PHMB Technical-Treated Rabbits[a]

Study days	Dose Group (mg/kg/day)			
	0	10	20	40
1-4	138	140	145	138
4-8	156	158	166	164
8-11	159	160	154	123**
11-14	153	150	143	120**
14-17	145	147	132	130
17-20	155	153	153	140
23-26	110	123	138	153**
26-30	96	109	117*	127*

[a]data taken from Table 7, page 41 of the report.

Prior to dosing (study days 1-4 and 4-8), food consumption was unaffected in PHMB treated rabbits. During the period of dosing (days 8-20), statistically significant reductions in food consumption were noted for rabbits at the 40 mg/kg/day dose level on days 8-11 and 11-14. These reductions amounted to decreases of 22% and 21% from control values, respectively. Food consumption was not affected during the latter part of the dosing period, but appeared to be part of a pattern of rebound which became statistically significant during the post-dosing period (study days 23-30). The rebound resulted in increases of 39% and 32% over control at the high dose for days 23-26 and 26-30, respectively, and an increase of 22% over control at the 20 mg/kg/day dose level. The increase at the 20 mg/kg/day dose level on days 26-30 may be the result of a decrease in control food consumption values for this time period, as there was no significant effect on food consumption at the 20 mg/kg/day dose level during the period of dosing.

5. Gross Pathology

On day 30 of the study, all surviving does were terminated by intravenous barbiturate overdose and a macroscopic post mortem examination was performed. The uterus from any animal without clear evidence of implantation was removed and stained with ammonium polysulphide to determine pregnancy status. For pregnant animals, the intact gravid uterus (minus ovaries and trimmed free of connective tissue) was removed and weighed.

The uterus was opened, and the number of live fetuses as well as early and late intrauterine deaths noted. The sex, length, and weight of fetuses were determined. All fetuses were examined internally for visceral abnormalities, sexed, eviscerated and fixed in methanol. After 24 hours in fixative, the head of each fetus was cut open and the brain examined for macroscopic abnormalities. Carcasses were then returned to methanol for subsequent processing and staining with Alizarin Red S. Stained fetal skeletons were examined for abnormalities and the degree of ossification assessed. Individual bones of the manus and pes were assessed and the result converted to a six point scale.

i) Gross Observations

A summary of findings in maternal rabbits was provided (Table 8, page 42 of the report). In those rabbits which died during the study (primarily at the high dose), there were findings in the cecum and stomach indicative of inappetance (gas filled/distended intestines, fluid contents in the intestine) as well as irritation (red areas, sloughed mucosa). In those rabbits surviving to study termination, there appeared to be few abnormal findings. There was the report of pale liver in one high dose rabbit, as well as red area in the glandular stomach of one high dose rabbit. One rabbit at the 20 mg/kg/day dose was reported with raised area and mucosal sloughing in the stomach.

ii) Histopathologic Observations

No histological data were provided on maternal tissues in this report.

iii) Organ Weights

The mean weight of the gravid uterus was provided for each dose group (Table 9, page 45 of the report). According to these data, there did not appear to be any significant differences in mean gravid uterine weight among the dose groups of rabbits. Mean weight ranged from 524±149 grams in the 40 mg/kg/day dose group to 553±100 grams in control.

iv) Cesarean Section Observations

Table 3: Cesarean Section Observations

Dose (mg/kg/day):	0	10	20	40
#Animals Assigned	20	20	20	20
#Animals Mated/ Inseminated	20	20	20	20
Pregnancy Rate (%)	95	100	90	100
Maternal Wastage #Died	1	0	0	6
#Died/pregnant	0	0	0	6
#Non pregnant	1	0	2	0
#Aborted	0	0	0	4
Whole Litter Resorptions[c]	0	0	0	1
Total # of litters	19	20	18	13
Total Corpora Lutea	225	223	204	163
Corpora Lutea/dam[d]	11.8	11.2	11.3	12.5
Total Implantations	192	195	176	127
Implantations/Dam[e]	10.11	9.75	9.78	9.77
Total Live Fetuses	181	187	153	119
Live Fetuses/Dam	9.53	9.35	8.50	9.15
Intra-Uterine Deaths Early	8	3	18	5
Late	3	5	5	3

Table 3a (cont.)

Dose (mg/kg/day):	0	10	20	40
Intrauterine deaths/Dam	0.6	0.4	1.3	0.6
Total Dead Fetuses		[no dead fetuses were reported]		
Dead Fetuses/Dam				
Mean Fetal Weight (gm)[f] (M + F)	40.4	41.4	43.5	42.3
% Preimplantation Loss(mean)[b]	14.7	12.6	13.7	22.1
% Postimplantation Loss (mean)[b]	5.7	4.1	13.1	6.3
Sex Ratio (percentage M/F)	43.3	48.9	55.1	54.2

[a]Data taken from Table 9 and Appendix 5, pages 45-46 and 235-238 of the report.
[b]values taken from Appendix 5; mean values in Table 9 were incorrect.

At the high dose level, a reduced number of litters were observed, as were reduced corpora lutea, reduced total implantations, and increased pre-implantation loss. However, as dosing did not begin until day 8 and implantation occurs prior to day 8 of gestation, thses effects are more likely due to maternal stress than treatment with test chemical. In addition, mean corpora lutea/dam and mean implantations/dam were unaffected by treatment, and post-implantation loss was also unaffected, further suggesting effects related to other than test chemical.

2. Developmental Toxicity

Fetal visceral and skeletal assessment have been described above. Summary of observations is made in the following table (Table 4):

TABLE 4

Developmental Toxicity of PHMB Technical[a]

Dose group (mg/kg/day)	0	10	20	40
Observations[a]				
#pups(litters) examined	181(19)	187(20)	153(18)	119(13)
#pups(litters) affected				
major external/visceral defects	3(2)	5(3)	3(3)	0(0)
minor external/visceral defects	5(5)	5(5)	6(6)	3(3)
external/visceral variants	0(0)	0(0)	0(0)	0(0)
major skeletal defects	0(0)	4(3)	3(3)	0(0)
minor skeletal defects	68(18)	68(19)	61(18)	49(10)
skeletal variants	160(19)	158(20)	126(18)	101(13)

[a] Data taken from Table 10, pages 47-48 of the report.

Definitions of "major" and "minor" were given in the report as follows:

major: "permanent structural or functional deviations considered likely to be incompatible with survival or rarely seen"

minor: "small, generally transient deviations considered compatible with survival."

As shown, the incidence of both major and minor defects and variants did not appear to be significantly altered as a result of treatment with PHMB. Examination of specific findings showed only a few instances where the incidence of a specific occurrence was statistically increased over control. This consisted of a non-ossified 5th sternebrae, found in 12 (10.1%) of fetuses from the 40 mg/kg/day dose group vs 6 (3.3%) in control, and a fused 4th and 5th sternebrae, found in 7 (5.9%) of fetuses and 6 (46.2%) of litters at the 40 mg/kg/day dose level, compared to 1 (0.6%) fetus and 1 (5.[illegible]%) litter in controls. There was also a numerical increase in the incidence of fetuses and litters with fused 3rd and 4th sternebrae at the 40 mg/kg/day dose [5(4.2%) of fetuses and 3

(23.1%) of litters at the high dose vs 1 fetus (0.6%) and 1 litter (5.3%) in controls.

Examination of the manus and pes (Table 13, page 61 of the report) showed no significant effect of PHMB treatment on ossification of these bones.

III. DISCUSSION

In the present study, the developmental toxicity of PHMB technical was assessed by oral administration of the chemical at doses of 0, 10, 20, and 40 mg/kg/day to pregnant female New Zealand White Rabbits on days 8-20 of gestation. Daily observations were made for maternal toxicity of PHMB technical, while body weights and food consumption were made at regular intervals during the study. Maternal rabbits were subject to macroscopic examination at study termination (day 30), while fetuses received complete macroscopic examination, including visceral and skeletal examinations.

Mortality was reported at the 40 mg/kg/day dose level, in which a total of 6 rabbits died intercurrently. Of these 6, four died as the result of abortion. This effect is considered to be a result of maternal toxicity, rather than a direct effect of PHMB. This is supported by the finding of no increase in post-implantation loss among maternal rabbits with live fetuses at study termination. At the 40 mg/kg/day dose level, clinical toxicity was also observed, and included signs of coldness (6/20 vs 0/20 in control), few feces (16/20 vs 7/20 in controls), no feces (6/20 vs 0/20 in controls), thin appearance (6/20 vs 0/20 in controls), and subdued behavior (3/20 vs 1/20 in controls).

Although no significant treatment-related effects were observed on body weight gain, a significant reduction in food consumption of 22% was observed during the first half of the dosing period at the 40 mg/kg/day dose level. A rebound effect was observed after the termination of dosing on study days 23-30, in which food consumption was increased approximately 39% over control.

The evaluation of observations made at cesarean section showed the following, observed at the 40 mg/kg/day dose level: reduced number of litters (13 vs 19 in control), reduced corpora lutea (163 vs 225 in control), reduced total implantations (127 vs 192 in control), and total live fetuses (119 vs 181 in control). Pre-implantation loss was also increased at the 40 mg/kg/day dose level (21.8 vs 13.1 in control). While these observations would suggest maternal toxicity, the possibility of developmental toxicity cannot be ruled out.

Examination of fetuses for visceral and skeletal abnormalities showed the following: Non-ossified 5th sternebrae, found in 12 (10.1%) of fetuses from the 40 mg/kg/day dose group vs 6 (3.3%) in control, and a fused 4th and 5th sternebrae, found in 7 (5.9%) of fetuses and 6 (46.2%) of litters at the 40 mg/kg/day dose level, compared to 1 (0.6%) fetus and 1 (5.3%) litter in controls. There was also a numerical increase in the incidence of fetuses and litters with fused 3rd and 4th sternebrae at the 40 mg/kg/day dose [5(4.2%) of fetuses and 3 (23.1%) of litters at the high dose vs 1 fetus (0.6%) and 1 litter (5.3%) in controls. Examination of the manus and pes (Table 13, page 61 of the report) showed no significant effect of PHMB treatment on ossification of these bones. Although visceral defects were noted in the study (Table 11, page 49), there was no apparent relationship of these findings to dose of PHMB, and thus these cannot be considered treatment-related abnormalities.

III.CONCLUSIONS

Administration of PHMB technical to pregnant female New Zealand White rabbits resulted in maternal toxicity at 40 mg/kg/day in the form of increased mortality and clinical toxicity. There was evidence of possible developmental toxicity of PHMB at 40 mg/kg/day, in the form of reduced number of litters and skeletal abnormalities (non-ossified 5th sternebrae, fused 3rd, 4th, and 5th sternebrae).

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Developmental toxicity NOEL = 20 mg/kg/day
Developmental toxicity LEL = 40 mg/kg/day (reduced no. of litters, skeletal abnormalities)

IV. CLASSIFICATION Core minimum

This study satisfies the guideline requirements (§83-3) for a developmental toxicity study in rabbits.

PHMB TOXR 010677

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Pages 15 through 16 are not included in this copy.

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