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

JUN 7 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Polyhexamethylene biguanide (PHMB, Baquacil): Review of a 21-Day Dermal Toxicity Study in Rats Submitted by the Registrant.

P.C. Code: 111801
Submission: S458169
MRID No: 430477-01
DP Barcode: D199277

FROM: Timothy F. McMahon, Ph.D., Pharmacologist 5/23/94
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TO: Kathryn Scanlon / PM 53
Special Review and Reregistration Division (7508W)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head J.M. Ioannou 5/23/94
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

and

Marcia Van Gemert, Ph.D., Branch Chief
Toxicology Branch II
Health Effects Division (7509C) M. Van Gemert 5/23/94

Registrant: Zeneca, Inc.

Action Requested: Review of a 21-Day Dermal Toxicity Study with PHMB in Rats submitted in support of reregistration of PHMB.

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Summary:

In a 21-Day Dermal Toxicity Study (MRID # 430477-01), PHMB (20.2% a.i.) was applied at dose levels of 0, 20, 60, and 200 mg/kg to the shorn backs of 5 male and female Alpk:APfSD (Wistar-derived) rats over the course of 30 days for a total of 21 applications of 6 hours duration each.

There was no evidence of systemic toxicity at any dose level in this study. Dermal toxicity was evident at the 60 mg/kg and 200 mg/kg dose levels in the form of erythema, edema, scabbing, acanthosis, and inflammatory cell infiltration in both sexes. Based on the data in this study, the systemic NOEL = 200 mg/kg/day for both sexes, and the systemic LEL > 200 mg/kg/day for both sexes. The dermal NOEL is = 20 mg/kg/day for both sexes, and the dermal LEL = 60 mg/kg/day for both sexes, based on the increased incidence of erythema, edema, and scabbing observed at this dose.

Although there was no evidence for systemic toxicity at any dose level tested in this study, higher dose levels were not tested due to the presence of severe dermal irritation at a dose level of 300 mg/kg in a preliminary study. Therefore, this study is classified as **core minimum data** and **satisfies** the guideline requirement (§82-2) for a 21-Day Dermal Toxicity Study in rats.

Reviewed by: Timothy F. McMahon, Ph.D. 5/23/94
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Y.M.I 5/23/94
Section I, Toxicology Branch II (7509C)

Data Evaluation Record

Study type: Twenty-one day dermal toxicity-rats
Guideline: 82-2

EPA ID Numbers: MRID number: 430477-01
P.C. Code: 111801
Submission: S458169
DP Barcode: D199277

Test material: Polyhexamethylene biguanide

Synonyms: PHMB; Baquacil

Study number(s): LR0559

Sponsor: Zeneca, Inc.

Testing Facility: Zeneca Central Toxicology Laboratory
Alderley Park, Macclesfield, Cheshire, UK

Title of report: PHMB: 21-Day Dermal Toxicity Study in the Rat.

Author(s): D Lees, A.M. Leah

Report issued: November 25, 1993

Executive Summary:

In a 21-Day Dermal Toxicity Study (MRID # 430477-01), PHMB (20.2% a.i.) was applied at dose levels of 0, 20, 60, and 200 mg/kg to the shorn backs of 5 male and female Alpk:APfSD (Wistar-derived) rats over the course of 30 days for a total of 21 applications of 6 hours duration each.

There was no evidence of systemic toxicity at any dose level in this study. Dermal toxicity was evident at the 60 mg/kg and 200 mg/kg dose levels in the form of erythema, edema, scabbing, acanthosis, and inflammatory cell infiltration in both sexes. Based on the data in this study, the systemic toxicity NOEL $\geq$ 200 mg/kg/day for both sexes, and the systemic toxicity LEL > 200 mg/kg/day for both sexes. The dermal

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toxicity NOEL is = 20 mg/kg/day for both sexes, and the dermal toxicity LEL = 60 mg/kg/day for both sexes, based on the increased incidence of erythema, edema, and scabbing observed at this dose.

Although there was no evidence for systemic toxicity at any dose level tested in this study, higher dose levels were not tested due to the presence of severe dermal irritation at a dose level of 300 mg/kg in a preliminary study. Therefore, this study is classified as **core minimum** data and satisfies the guideline requirement (§82-2) for a repeated dermal toxicity study in rats.

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I. MATERIALS AND METHODS

A. Test Material: Polyhexamethylene biguanide
purity: 20.2%
description: very faint yellow liquid
CTL ref. #: Y00156/008

B. Vehicle: none

C. Test Animals: Species: Alpk:APfSD (Wistar-derived)
Source: Barriered Animal Breeding Unit, Zeneca Pharmaceuticals, UK
Age: males: approximately 9-10 weeks; females: 11-15 weeks
Weight (at beginning of study): males, 304.9±9.5g; females, 262.2±8.08g

D. Animal Husbandry:

Rats were housed individually in suspended stainless steel wire mesh cages (solid sides, meshed front, back, and floor) in an animal room designed to maintain a temperature of 21 ± 2 °C and humidity of 55±15%. Light cycle was stated as 12 hours light/dark. Rats had free access to food (Portion Combined Diet, Special Diet Services Ltd) and tap water. Acclimation period was one week, during which rats were randomly allocated to cages. All animals were also observed prior to study initiation to ensure they exhibited normal activity and were also physically normal.

E. Experimental Design and Dosing:

The main study was preceded by a preliminary study in which groups of 2 male and female rats were given repeated dermal applications of 200, 300, 400, or 600 mg PHMB/kg. According to the report (page 13), there were no significant signs of toxicity at any dose level, but slight skin irritation was observed following 10 applications of 200 mg/kg PHMB, and extreme skin irritation was observed following 21, 11, or 8 applications of 300, 400, or 600 mg/kg PHMB, respectively. Based on these results, doses of 20, 60, and 200 mg/kg were selected for the main study.

In the main study, rats (5/sex/dose) received dermal application of test material at doses of 0, 20, 60, and 200 mg/kg/day, 6 hours per day, for five days a week. A total of 21 applications were made, excluding weekends. Prior to dosing, animals were acclimated to plastic collars used during the actual study to prevent oral contamination following each dermal application.

Sixteen to 24 hours prior to application of test material, hair was removed with a pair of veterinary clippers from an area approximately 10cm x 5cm on the dorso-lumbar region of each animal. Test material was spread evenly onto the shaved backs using disposable syringes or pipettes as appropriate. Differences in dose levels were achieved by altering the volume applied to each rat. The volume of the dose was calculated for each animal according to wieght at time of dosing.

Test material was kept in contact with the skin for approximately 6 hours using occlusive dressings, which consisted of a 4cm x 6cm gauze patch covered by a patch of

plastic film. This was held in place by using adhesive bandage which in turn was secured by 2 pieces of PVC tape wrapped around the animal. After each exposure period, the dressings were carefully cut, removed, and discarded. Skin was cleaned free of any residual test material using clean swabs of absorbent cotton wool soaked in clean warm water. Clean tissue paper was then applied gently to dry the area. The plastic collars mentioned earlier were fitted to the animals in between exposures to prevent oral contamination.

F. Statistical Analysis:

Statistical significance was analyzed using analysis of variance carried out with the GLM procedure in SAS. Differences from control were tested by comparing each treatment group least-squares mean with the control group least-squares mean using a 2-sided Student's t-test, based on the error mean square in the analysis. The 0.05 level of probability was selected as the criterion of statistical significance.

G. Compliance:

A signed Statement of No Data Confidentiality Claims was provided.

A signed Compliance with Good Laboratory Practices statement was provided.

A signed Quality Assurance Statement was provided.

II. OBSERVATIONS and RESULTS:

A. Clinical Signs and Mortality:

Detailed clinical observations were recorded prior to dosing and after decontamination, and once daily on the days when animals were not dosed. Results of clinical observations were made in Table 2 of the report, beginning on page 33. There were no apparent signs of systemic toxicity at any dose level tested.

B. Skin irritation:

Signs of skin irritation were present in a dose-related manner. Erythema, edema, scabbing, and small scattered scabs were the most prevalent signs in both sexes, and were found mainly at the 60 and 200 mg/kg dose levels. These observations are summarized below:

Table 1

Clinical Observations from Repeated Dermal Application of PHMB[a]

	Males		Females	
	60 mg/kg	200 mg/kg	60 mg/kg	200 mg/kg
erythema				
no. obs.	3	140	18	199
no. rats	2	5	4	5
days	29-30	4-30	19-28	2-30
edema				
no. obs.	1	115	0	149
no. rats	1	5	0	5
days	29-29	4-30	0	4-30
scabbing				
no. obs.	0	24	0	49
no. rats	0	4	0	3
days	0	17-30	0	9-30
small scabs, scattered				
no. obs.	3	95	21	149
no. rats		4	3	5
days	2.. 4	3-30	2-30	2-30

[a]data taken from pages 33-47 of the report.

As shown, signs of erythema, edema, and scabbing were mildly evident at the 60 mg/kg dose level, but were more prominent at the 200 mg/kg dose level in both sexes. According to the report, the signs of moderate irritation seen at this dose persisted in most animals until the end of the study, whereas at the 60 mg/kg dose level, the signs of irritation had completely regressed in all but 3 animals by the end of the study.

C. Body weight:

Body weight data were collected for each animal on study before application of test material. Results were presented in Table 3, pages 49-54 of the report. The data indicated no statistically significant differences in group mean body weight in treated rats vs control in either male or female rats. It was noted that on day 24, a significant difference in group mean body weight was observed in control male rats vs treated rats at the 200 mg/kg dose. The reason for this difference could not be ascertained, but was not believed to be compound related.

Body weight gain in male rats for the treatment period (30 days) showed that treated

groups of rats gained more weight vs control. Weight gain for the 20, 60, and 200 mg/kg dose groups was 62.6, 66.0, and 63.0 grams, respectively vs 43.0 grams in control. In female rats, weight gain was approximately equal among control and treated groups (39.2, 30.2, 44.4, and 28.6 grams in control, 20, 60, and 200 mg/kg dose groups, respectively).

D. Food consumption:

Food consumption was calculated on a weekly basis for all animals. Summary data on food consumption were presented in Table 4, pages 55-56 of the report. These data indicated no significant differences in food consumption in treated male and female rat at any dose level vs control.

E. Clinical Pathology:

At termination, all rats were bled by cardiac puncture, samples collected in tubes containing EDTA, and the following **hematology** parameters measured using a TECHNICON H1:

_x_ total leucocyte count*
_x_ erythrocyte count*
_x_ hemoglobin*
_x_ hematocrit*
_x_ platelet count*
_-_ total plasma protein*
_x_ leukocyte differential*
_x_ mean corpuscular HGB
_x_ mean corpusc. HGB conc.*
_x_ mean corpusc. volume
_-_ reticulocytes

*EPA guideline requirement

In addition to the above, further blood smaples were collected into tubes containing 0.1M trisodium citrate as anticoagulant and prothrombin and kaolin-cephalin times measured using an ACL 200. Blood films were also prepared and a differential leukocyte count and red cell morphological assessment performed in control and high dose rats.

A summary of hematological changes observed in this study was given in the report (Table 5, pages 57-61). There were no treatment-related alterations in any of the hematological parameters measured in male or female rats.

Clinical Chemistry: The following CHECKED parameters were measured using a KONE specific analyzer:

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

_X_ AST(SGPT)*
_X_ ALT(SGOT)*
_X_ alkaline phosphatase
_X_ creatine kinase
_-_ lactate dehydrogenase
_-_ sorbitol dehydrogenase
_X_ gamma glutamyl trans-peptidase
_-_ plasma cholinesterase
_-_ brain cholinesterase

_X_ calcium*
_X_ phosphorous*
_X_ sodium*
_X_ potassium*
_X_ chloride*

*EPA guideline requirement "-" not examined

Clinical chemistry data were summarized in Table 6, pages 61-64 of the report. These data indicated only a few alterations in clinical chemistry, such as a 30% increase in alkaline phosphatase activity in high dose females vs control, and a 10-13% increase in aspartate aminotransferase in all dosed female rats vs control. However, a corresponding effect was not observed in male rats. Thus, there is most likely no treatment-related significance to these findings.

3) **Urinalysis**: Urinalysis was not performed in this study.

F. Anatomic and Histologic Pathology:

Animals surviving to study termination were killed by exsanguination under terminal anesthesia using halothane. Testes, kidneys, and liver were weighed. Paired organs were weighed together.

The following list of tissues were obtained from all animals and fixed in 10% neutral buffered formalin, except for skin, testes, and epididymides, which were fixed in Bouin's solution.

kidneys	skin (untreated)	any other tissue
liver	skin (treated)	showing macroscopic
adrenal	testes with epididymides	abnormalities
brain		

Organ weight data were summarized in Table 7, pages 65-67, while macroscopic observations were recorded in Table 8, page 68 of the report. The only statistically significant observation with regard to organ weight was a significant increase in the paired weight of the kidneys in male rats at the 200 mg/kg dose(3.20±0.32g) vs control male kidneys (2.92±0.27g). Although an increase of 11% was noted in liver weight among high dose male rats (16.3±1.8g) vs controls (14.6±2.0g) this was not statistically significant.

Macroscopic data, as summarized in the report, showed no macroscopic abnormalities other than those previously mentioned with regard to skin.

Microscopic examination of tissues (Table 9, pages 69-70 of the report) showed abnormalities with respect to the skin, including acanthosis in male rats at the 60 and 200 mg/kg dose, and inflammatory cell infiltration in both male and female rats primarily at the 200 mg/kg dose level. The acanthosis in male rats was present in one rat at the 60 mg/kg dose, and was graded as minimal. At the 200 mg/kg dose level, acanthosis was observed in all 5 male rats, and was graded as minimal in 3 rats, slight in 1 rat, and moderate in 1 rat. Inflammatory cell infiltration was present in 4 rats of both sexes at the 200 mg/kg dose. In males, 2 rats were given minimal grades for this lesion, while the other 2 were graded as slight for this lesion. In females, 1 rat was graded as minimal for inflammatory cell infilatration, 2 as slight, and 1 as marked.

III. DISCUSSION

In this study, the toxicity of PHMB from repeated dermal application to male and female Sprague-Dawley rats was assessed. Technical PHMB (20.2% a.i.) was administered dermaliy to groups of 5 male and 5 female rats at dose levels of 0, 20, 60, and 200 mg/kg/day. Rats received a total of 21 doses of test chemical for 6 hours per day, five days per week over a 30 day test period.

Changes in absolute body weight, body weight gain, and food consumption were monitored over the course of this study, as well as any dermal reaction and clinical toxicity resulting from administration of PHMB technical. Blood samples were obtained from all rats at study termination for assessment of hematology and clinical chemistry in treated vs control rats. Organ weights were obtained at necropsy for the liver, kidneys, and testes with epididymides, and were also examined both macroscopically and microscopically for abnormalities.

The few effects noted in this study were primarily related to skin reactions to treatment with PHMB. This would be expected in light of the existing toxicology database showing PHMB technical to be both a skin irritant and skin sensitizer. The effects noted in this study are summarized below:

1) An increase in the incidence of erythema, edema, and scabbing in male and female rats at the 60 and 200 mg/kg dose levels.

2) A 30% increase in alkaline phosphatase activity in high dose female rats vs control at study termination.

3) A significant increase in the paired weight of the kidneys in high dose male rats vs control.

4) An increase in the incidence of acanthosis and inflammatory cell infiltration in male and female rats primarily at the 200 mg/kg dose level.

Based upon the above effects, a systemic effect level was not established in this study, i.e. is > 200 mg/kg. A dermal effect level for PHMB technical can be established at the 60 mg/kg dose level based on the increase in erythema, edema, and scabbing at this dose. Testing at higher doses is not necessary due to severe dermal irritation observed at a dose of 300 mg/kg in the preliminary study with PHMB.

IV. CLASSIFICATION - core minimum

This study satisfies the guideline requirement (§82-2) for a repeated dermal toxicity study in rats.