CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

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CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
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EXECUTIVE SUMMARY

On April 9, 2003, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of PHMB.

Dr. Jonathan Chen of the Antimicrobials Division presented the chronic toxicity/carcinogenicity studies in Wistar rats and C57B1/10JfCD-1/Alpk and Alderley Park mice by describing the experimental design; reporting on survival and body weight effects treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, and the adequacy of the dose levels tested; and presenting the weight of the evidence for the carcinogenicity of PHMB.

PHMB was administered in the diet: 1) to male and female Wistar rats (52 rats/sex) at dose levels of 0, 200, 600, or 2000 ppm (0, 12.2, 36.3, or 126.1 mg/kg/day for males; 0, 14.9, 45.3, or 162.3 mg/kg/day for females) of PHMB for 105 weeks; and 2) to C57B1/10J$_t$CD-1/Alpk mice (55/sex/group) at 0, 400, 1,200 or 4,000 ppm (equivalent to 55, 167, or 715 mg/kg/day for males and 69, 217, or 856 mg/kg/day for females) for 2 years.

In addition, four groups of specific pathogen free (50M + 50F) Alderley Park Mice received dermally 0.3 mL of test material at doses of 0 (solvent in methanol), 0.6 mg (0.2% PHMB in ethanol), 6.0 mg (2% PHMB in ethanol), or 30.0 mg (10% PHMB in ethanol) per mouse per day for five days a week for 80 weeks. The treatment dosages are approximately equivalent to 0, 15, 150 and 750 mg/kg/day of 20% PHMB solution.

The CARC concluded that PHMB showed evidence of carcinogenicity based on the following:

Rat - Oral

- There was no treatment-related increase in any tumors in male Wistar rats.

- *Vascular Tumors*: Female Wistar rats from the Pathology Working Group (PWG) diagnoses had significant increasing trends in liver hemangiomas and liver hemangiosarcomas, both at $p < 0.05$. There was also a significant increasing trend for hemangiomas and/or hemangiosarcomas at all sites combined at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 2000 ppm dose group with the controls for liver hemangiomas and hemangiomas and/or hemangiosarcomas at all sites combined, both at $p < 0.05$. The incidence of hemangiomas and hemangiosarcomas for all sites combined was 2/42 (5%), 1/42 (2%), 3/40 (8%), and 6/35 (17%) for the 0, 200, 600, and 2000 ppm dose groups, respectively. Historical control data regarding hemangiosarcomas shows the range of hemangiosarcomas by tissue in rats was 0-1.9%. **The CARC considered the tumor response at the high dose (17% for the combined re-read of liver and original diagnoses at other sites) to be treatment-related and driven by the increase in hemangiomas when all sites are combined.**

- *Adequacy of Dosing:* The CARC concluded that dosing at the highest dose (2000 ppm) was considered to be adequate, but not excessive, for both male and female rats. In high dose females, this was based on increased mortality and a reduction in body weight of 5-8% throughout the study. In high-dose males, body weights were significantly reduced (4-6%) through week 79. Food utilization (g growth/100 g feed) for the first 12 weeks decreased significantly (↓7-8%) in both sexes at 2000 ppm.

Mouse - Oral

- *Vascular Tumors:* Male C57B1/10J$_f$CD-1/Alpk mice from the PWG diagnoses had significant increasing trends in hemangiomas, hemangiosarcomas, and combined hemangiomas and hemangiosarcomas, all at $p<0.01$. There were also significant differences in the pair-wise comparisons of the 4000 ppm dose group with the controls, for hemangiomas ($p<0.01$), hemangiosarcomas ($p<0.05$), and combined hemangiomas and/or hemangiosarcomas ($p< 0.01$). The incidence of hemangiomas was 2/55 (4%), 3/55 (5%), 4/55 (7%), 11/53 (21%), for the 0, 400, 1200, and 4000 ppm dose groups, respectively. The incidence of hemangiosarcomas was 5/55 (9%), 4/55 (7%), 6/55 (11%), and 12/53 (23%) for the 0, 400, 1200, and 4000 ppm dose groups, respectively. The incidence of combined hemagiomas and hemangiosarcomas was 6/55 (11%), 6/55 (11%), 9/55 (16%), and 20/53 (38%) for the 0, 400, 1200, and 4000 ppm dose groups, respectively. For hemangiosarcomas, the tumor incidence in the 4000 ppm dose group (23%) exceeded the historical control range (1.8-18.3%). **The CARC considered the increase in vascular tumors (hemangiomas, hemangiosarcomas, and combined) at the high dose (which is considered to be excessive) to be treatment-related. Although not statistically significant, the tumor response (9/55 [16%] for the combined hemangiomas and hemangiosarcomas) in mice at the mid-dose of 1200 ppm is considered treatment-related since this tumor type was also seen in female mice (orally and dermally) and female rats.**

- *Vascular Tumors:* Female C57B1/10J$_f$CD-1/Alpk mice from the PWG diagnoses had significant increasing trends in hemangiomas ($p<0.05$), hemangiosarcomas ($p<0.05$), and combined hemangiomas and hemangiosarcomas ($p<0.01$). There was also a significant difference ($p< 0.05$) in the pair-wise comparison of the 4000 ppm dose group with the controls, for combined hemangiomas and/or hemangiosarcomas. The incidence of combined hemangiomas and/or hemangiosarcomas was 8/54 (15%), 5/53 (9%), 7/54 (13%), and 15/49 (31%) for the 0, 400, 1200, and 4000 ppm dose groups, respectively. For hemangiosarcomas, the tumor incidence in the 4000 ppm dose group (31%) exceeded the historical control range (0-10.9%). There are no historical control data for hemangiomas by tissue. **The CARC considered the combined vascular tumors at the high dose (which is considered to be excessive) to be treatment-related. There were no increases in these tumors at doses which were not considered excessive.**

- *Rectal-Anal Junction Tumors:* In male mice there was a significant increasing trend at $p < 0.01$, and a significant difference in the pair-wise comparison of the 4000 ppm dose group with the

controls at $p < 0.05$, for rectal-anal junction squamous cell carcinomas. The incidence of rectal-anal junction tumors was 0/45, 0/45, 0/45, and 5/48 for the 0, 400, 1200 and 4000 ppm dose groups, respectively. In female mice there was a significant increasing trend at $p < 0.01$ and a significant difference in the pair-wise comparison of the 4000 ppm dose group with the controls at $p < 0.01$, for rectal-anal junction squamous cell carcinomas. The incidence of rectal anal junction tumors was 0/42, 0/43, 0/43, 8/28 for the 0, 400, 1200 and 4000 ppm dose groups, respectively. **The CARC considered the carcinomas at the high dose (which is considered to be excessive) in both males and females to be treatment-related but suggestive of a local irritant effect. These tumors did not contribute to the CARC's weight-of-the-evidence analyses.**

- *Adequacy of Dosing:* Dosing was considered to be excessive at the high dose of 4000 ppm based on decreased overall body weight gains of 50% in males and 32% in females at termination. In addition, decreased body weight gains of 33% in males and 19% in females were seen at 13 weeks. The study indicated that the animals at the 4000 ppm dose group had increased food consumption ($p<0.05$ or 0.01) from approximately week 12 through termination in males (↑7-29%) and females (↑7-26%).

  Dosing was considered to be adequate, but not excessive, in both males and females at the mid-dose of 1200 ppm based on decreased overall body weight gains (males, 7%; females, 2%), increases in hematology parameters, and non-neoplastic histopathological changes in the gall bladder, liver, and rectal-anal junction.

Mouse - Dermal

- *Vascular Tumors:* Female Alderley Park mice had significant increasing trends in liver angiosarcomas ($p < 0.01$) and vascular tumors from all sites combined ($p < 0.05$). There was also a significant difference in the pair-wise comparison of the 30 mg dose group with the controls for liver angiosarcomas at $p < 0.05$. The incidence of liver angiosarcomas was 0/41, 0/45, 0/40, and 2/35 for the 0, 0.6, 6.0, and 30 mg dose groups, respectively. **The CARC considered the tumor increase at the high dose to be equivocal since the tumors were only seen at an excessive dose. Vascular tumors were not seen at lower doses.**

  There was no treatment-related increase in any tumors in male Alderley Park mice.

- *Adequacy of Dosing*: Dosing was considered to be excessive at the high dose (750 mg/kg/day of 20% PHMB equivalent) in both sexes due to increased mortality (78%, high dose, versus 33% and 28% in the male and female controls, respectively) and decreased body weight gain. Overall body weight gain decreases of 45% and 17% were seen in males and females, respectively. No treatment-related effects were noted at the mid-dose level of 150 mg/kg/day of 20% PHMB equivalent.

Mutagenicity

- There is no mutagenicity concern.

Structure Activity Relationship

- No appropriate structural analogs could be located for comparison purposes. In general, with the exception of exposure via the inhalation route, high molecular weight (MW) polymers are not expected to be of significant carcinogenic concern because of poor bioavailability. PHMB has indeed been shown to have low bioavailability. However, SAR consideration suggests that there is a likelihood that the lower MW fractions of PHMB may be metabolized to polyamines in analogy to the metabolism of arginine (a guanidine-containing amino acid) to form biogenic polyamines such as putrescine, spermidine and spermine. There is strong evidence that these polyamines are readily taken up by cells and may play an important role in promoting skin and colon carcinogenesis by serving as ingredients to support neoplastic growth. A metabolism study submitted by the company showed that low MW fractions of PHMB can indeed be metabolized in the rat; however, the metabolites have not yet been identified.

Mode of Action

- No mode of action studies were available.

In accordance with the EPA *Draft Guidelines for Carcinogen Risk Assessment* (July 1999), the CARC classified PHMB into the category **"Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential"** by the oral and dermal routes. The quantification of human cancer risk is not required.

I. INTRODUCTION

On April 9, 2003, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Program met to evaluate the carcinogenic potential of PHMB. This was the first time this compound was assessed for carcinogenicity by the CARC.

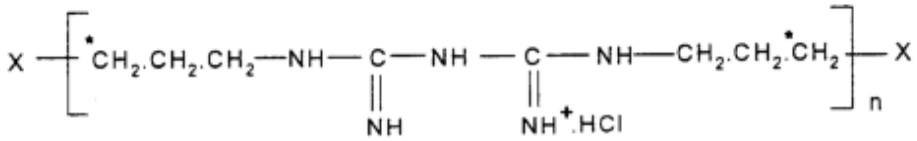
II. BACKGROUND INFORMATION

PHMB, also known as Baquacil; Baquacil SB; Cosmoquil CQ; polihexanide; polyhexamethylbiguanide; poly(hexamethylenebiguanide) hydrochloride; Vantocil 1B; or polyhexamethylene biguanide. The CAS Number of this chemical is 32289-58-0. The PC Code of PHMB is 111801. PHMB is a group of polymers (see Figure 1) that has been used as an antimicrobial agent in a wide variety of applications including oil-in-water and water-in-oil emulsions, industrial reagents, silicone systems, cellulose solutions and oil recovery systems. Now, PHMB is primarily used as a non-chlorinated antimicrobial agent in swimming pool and spa facilities.

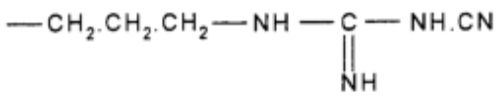
On 12/18/2000, 01/25/2001, 01/30/2003 and 02/06/2003, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicology data base of **PHMB,** established the Reference Doses (RfDs) and selected the toxicological endpoints for dietary as well as occupational exposure risk assessments.

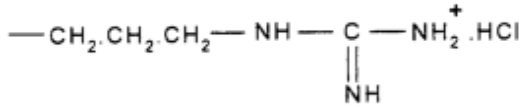
Figure 1: Chemical Structure of PHMB

n= 1-40



Where X = $-CH_2.CH_2.CH_2-NH_2^+.HCl$

or 

or 

III. EVALUATION OF CARCINOGENICITY STUDIES

1. 2-Year Chronic Toxicity and Carcinogenicity Study in Rats (1996)

Reference:

Horner, S. A. (1996) Polyhexamethylene Biguanide: Two Year Feeding Study in Rats. Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID: Report No. CTL/P/4663, Study No. PR0936, June 5, 1996. MRID 44059301. Unpublished.

Busey, W. M. (1996) Polyhexamethylene Biguanide (PHMB): Two Year Feeding Study in Rats. Pathology Working Group Peer Review of Proliferative Vascular Lesions in Male and Female Rats. Zeneca CTL, Alderley Park, Macclesfield, Cheshire, UK. Study No. PR0936, Report No. CTL/C/3172, May 13, 1996. MRID 44042801. Unpublished.

A. Experimental Design

A combined chronic toxicity/oncogenicity study in Alpk:APfSD Wistar rats was conducted by Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England, for Zeneca Specialities, Wilmington, Delaware and dated June 5, 1996 (Report No. CTL/P/4663, Study No. PR0936, MRID No. 44059301). **A Pathology Working Group (PWG) peer review reevaluated the vascular lesions of this study in a report dated May 13, 1996 (Report No. CTL/C/3172, MRID No. 44042801). Both the original and the reread diagnoses are presented in this document.**

The study design allocated groups of 52 rats per sex to dose levels of 0, 200, 600, or 2000 ppm (0, 12.2, 36.3, or 126.1 mg/kg/day for males; 0, 14.9, 45.3, or 162.3 mg/kg/day for females) of PHMB for 105 weeks. An additional 12 rats per sex per dose were designated for interim sacrifice at week 52.

B. Discussion of Tumor Data

Survival Analysis

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of PHMB in male Alpk:APfSD Wistar rats. Female Alpk:APfSD Wistar rats had a significant increasing trend in mortality with increasing doses of PHMB ($p<0.01$) and a significant difference in the pair-wise comparison of the 2000 ppm dose group ($p < 0.05$) with the control group (Memo, L. Brunsman, 12/03/02, TXR No. 0051368). Tables 1 and 2 show the mortality results.

Table 1. PHMB - 1996 Alpk:APfSD Wistar Rat Study

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-52	53[i]	53-78	79-105[f]	Total
0	0/64 (0)	0/64 (0)	12/64	5/52 (10)	27/47 (57)	32/52 (62)
200	0/64 (0)	3/64 (5)	12/61	7/49 (13)	26/42 (58)	36/52 (69)
600	0/64 (0)	2/64 (3)	12/62	10/50 (19)	20/40 (48)	32/52 (62)
2000	3/64 (5)	2/61 (3)	10/59	5/49 (10)	22/44 (48)	32/54 (59)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 53.

[f]Final sacrifice at week 105.

( )Percent.
Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. PHMB - 1996 Alpk:APfSD Wistar Rat Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-52	53[I]	53-78	79-105[f]	Total
0	0/64 (0)	0/64 (0)	12/64	6/52 (12)	19/46 (41)	25/52 (48)**
200	0/64 (0)	0/64 (0)	12/64	5/52 (10)	15/47 (32)	20/52 (38)
600	1/64 (2)	1/63 (2)	12/62	7/50 (14)	14/43 (32)	23/52 (44)
2000	1/64 (2)	4/63 (6)	12/59	6/47 (12)	21/41 (47)	32/52 (62)*

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 53.

[f]Final sacrifice at week 105.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Tumor Analysis

The study pathologist and study peer reviewer determined that there were 3 hemangiosarcomas of the liver in 2000 ppm females. Benign hemangiomas were not observed. The increased incidence of hemangiosarcomas in females gave positive results in trend analyses ($p<0.05$). A single observation of benign hemangioma was made in each of the control and high dose males; no hemangiosarcomas were observed in males. A Pathology Working Group (PWG) was convened in 1996 to confirm the diagnoses of the vascular neoplasms (MRID 44042801). The PWG determined that there were 2 hemangiomas and 1 hemangiosarcoma in 2000 ppm females and 2 hemangiomas in 2000 ppm males. The PWG concluded that the findings of vascular neoplasms in high dose females were incidental. However, the report of the PWG consensus indicated that no hemangiosarcoma or hemangioma had been observed in female controls in 18 studies with the same strain of rat. Dr. J.M. Pletcher, EPA's consulting pathologist, confirmed the validity of the PWG report (TXR No. 0052000).

There were no compound-related tumors observed in male rats. Female rats from the original study diagnoses had significant increasing trends in liver hemangiosarcomas and hemangiomas and/or hemangiosarcomas at all sites combined, both at $p < 0.05$. There was also a significant difference in the pair-wise comparison of the 2000 ppm dose group with the controls for liver hemangiosarcomas at $p < 0.05$.

Female rats from the PWG diagnoses had significant increasing trends in liver hemangiomas and liver hemangiosarcomas, both at $p < 0.05$. There was also a significant increasing trend for hemangiomas and/or hemangiosarcomas at all sites combined at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 2000 ppm dose group with the controls for liver hemangiomas and hemangiomas and/or hemangiosarcomas at all sites combined, both at $p < 0.05$ (Memo, L. Brunsman, 12/03/02, TXR No. 0051368).

The statistical analyses of the female rats were based upon Peto's prevalence test. Tables 3 and 4 show the tumor analyses results.

Historical control data regarding hemangiosarcomas from studies using the Alpk:ApfSD Wistar rats and conducted by the Syngenta Central Toxicology Laboratory (Alderley Park, UK) are provided (MRID 457108-08). The total number of rats with hemangiosarcomas recorded, irrespective of site, are summarized in Table 5. The occurrence of hemangiosarcomas by tissue in rats is shown in Table 6. The historical control range for hemangiosarcomas was 0-1.9% for both male and female rats.

Table 3. PHMB - 1996 Alpk:APfSD Wistar Rat Study

ORIGINAL STUDY DIAGNOSES

Female Vascular Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	200	600	2000
Liver Hemangio-sarcomas (%)	0/42 (0)	1/42 (2)	0/40 (0)	3[a]/35 (9)
p =	0.0107*	0.1284	-	0.0338*
Other Sites (NOT LIVER) Hemangiomas (%)	1/42 (2)	1/41 (2)	3/39 (8)	3[b]/34 (9)
p =	0.0733	0.4208	0.1430	0.0748
Other Sites (NOT LIVER) Hemangio-sarcomas (%)	1[c]/40 (2)	0/40 (0)	0/35 (0)	0/28 (0)
p =	-	-	-	-
Combined (%)	2/42 (5)	2/42 (5)	3/40 (8)	6/35 (17)
p =	0.0234*	0.3094	0.2804	0.0575

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First liver hemangiosarcoma observed at week 91, dose 2000 ppm.

[b]First other sites (NOT LIVER) hemangioma observed at week 92, dose 2000 ppm.

[c]First other sites (NOT LIVER) hemangiosarcoma observed at week 95, dose 0 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. PHMB - 1996 Alpk:APfSD Wistar Rat Study

Female Vascular Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	200	600	2000
PWG				
RE-READ OF LIVER VASCULAR TUMORS				
Heman-giomas (%)	0/42 (0)	0/42 (0)	0/40 (0)	2[a]/35 (6)
p =	0.0107*	-	-	0.0808*
Hemangio-sarcomas (%)	0/27 (0)	0/32 (0)	0/29 (0)	1[b]/20 (5)
p =	0.0232*	-	-	0.1226
ORIGINAL DIAGNOSES OF VASCULAR TUMORS AT OTHER SITES				
Hemangiomas (%)	1/42 (2)	1/41 (2)	3/39 (8)	3[c]/34 (9)
p =	0.0733	0.4208	0.1430	0.0748
Hemangio-sarcomas (%)	1[d]/40 (2)	0/40 (0)	0/35 (0)	0/28 (0)
p =	-	-	-	-
COMBINED PWG RE-READ OF LIVER AND ORIGINAL DIAGNOSES AT OTHER SITES				
Combined (%)	2/42 (5)	1/42 (2)	3/40 (8)	6/35 (17)
p =	0.0079**	-	0.2869	0.0344*

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First liver hemangioma observed at week 91, dose 2000 ppm.
[b]First liver hemangiosarcoma observed at week 105, dose 2000 ppm.
[c]First other sites (NOT LIVER) hemangioma observed at week 92, dose 2000 ppm.
[d]First other sites (NOT LIVER) hemangiosarcoma observed at week 95, dose 0 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Historical control data regarding hemangiosarcomas from studies using the Alpk:ApfSD Wistar rats and conducted by the Syngenta Central Toxicology Laboratory (Alderley Park, UK)

TOTAL NUMBER OF RATS WITH HEMANGIOSARCOMA RECORDED, IRRESPECTIVE OF SITE			
Study	Start Date	Male	Female
Study 1	1984/02	0/104 (0.0%)	0/104 (0.0%)
Study 2	1984/10	1/52 (1.9%)	0/52 (0.0%)
Study 3	1985/02	0/52 (0.0%)	0/52 (0.0%)
Study 4	1985/08	0/52 (0.0%)	0/52 (0.0%)
Study 5	1986/10	0/52 (0.0%)	0/52 (0.0%)
Study 6	1987/02	0/52 (0.0%)	1/52 (1.9%)
Study 7	1987/11	0/52 (0.0%)	0/52 (0.0%)
Study 8	1988/06	0/52 (0.0%)	0/52 (0.0%)
Study 9	1989/09	1/56 (1.8%)	1/56 (1.8%)
Study 10	1990/04	0/52 (0.0%)	0/52 (0.0%)
Study 11	1990/07	1/52 (1.9)	1/52 (1.9%)
Study 12	1992/04	1/52 (1.9%)	0/52 (0.0%)
Study 13	1992/05	0/52 (0.0%)	0/52 (0.0%)
Study 14	1992/11	1/52 (1.9%)	1/52 (1.9%)
Study 15	1994/05	0/52 (0.0%)	0/52 (0.0%)
Study 16	1994/11	1/52 (1.9%)	0/52 (0.0%)
Study 17	1995/01	0/52 (0.0%)	0/52 (0.0%)
Study 18	1995/02	1/52 (1.9%)	0/52 (0.0%)
Study 19	1996/04	0/52 (0.0%)	0/52 (0.0%)

Table 6. Historical control data regarding hemangiosarcomas from studies using the Alpk:ApfSD Wistar rats and conducted by the Syngenta Central Toxicology Laboratory (Alderley Park

OCCURRENCE OF HEMANGIOSARCOMAS IN RATS, BY TISSUE			
Study	Start Date	Male	Female
Study 1	1984/02		
Study 2	1984/10	kidney	
Study 3	1985/02		
Study 4	1985/08		
Study 5	1986/10		
Study 6	1987/02		vagina
Study 7	1987/11		
Study 8	1988/06		
Study 9	1989/09	lymph node (mesenteric)	limb
Study 10	1990/04		
Study 11	1990/07	jejunum	lymph node (mesenteric)
Study 12	1992/04	lymph node (mesenteric)	
Study 13	1992/05		
Study 14	1992/11	kidney	lymph node (mesenteric)
Study 15	1994/05		
Study 16	1994/11	spleen	
Study 17	1995/01		
Study 18	1995/02	lymph node (mesenteric and thymic)	
Study 19	1996/04		

C. Non-Neoplastic Lesions

In addition to liver hemangiomas and hemangiosarcomas, the liver is also the target organ in males and females. Plasma alkaline phosphatase activity was elevated significantly over controls ($p<0.01$) in females from the main study dosed at 2000 ppm (↑43-74%). In 2000 ppm males the enzyme was significantly increased by 36% and 27% at weeks 14 and 27, respectively. In females at 2000 ppm, absolute liver weight was reduced significantly ($p<0.05$) by 11%. In 2000 ppm males, microscopic observations of liver hepatocyte fat and spongiosis were increased 44% and 22% over controls, respectively. Corresponding increases in these lesions were not seen in females. There were no corroborating gross pathology findings of the liver abnormalities.

D. Adequacy of the Dosing for Assessment of Carcinogenicity

The dosing was considered to be adequate, but not excessive, for both male and female rats. In the rat study at the high dose (2000 ppm) there was significantly increased mortality in females (62% vs. 48% in controls). Body weights were significantly reduced by 5-8% in high-dose females throughout the study. In high-dose males, body weights were significantly ($p<0.01$ or 0.05) lower than controls (↓4-6%) through week 79. Food utilization (g growth/100 g feed) for the first 12 weeks decreased significantly ($p<0.01$) vs controls (↓7-8% in both sexes at 2000 ppm). Plasma alkaline phosphatase activity was significantly elevated 43-74% over controls in 2000 ppm females from the main study at the 27, 53, 79, and 105-week intervals. In males at 2000 ppm, alkaline phosphatase activity was increased significantly by 36% and 27% during weeks 14 and 27, respectively.

2. 2-Year Oncogenicity Study in Mice (1996)

Reference:

Milburn G. M. (1996) Polyhexamethylene Biguanide: Two Year Oncogenicity Study in Mice. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Laboratory Project ID: PM0937. June 21, 1996. MRID 44074201. Unpublished.

Mann, P. (2002) Polyhexamethylene Biguanide (PHMB): Two Year Oncogenic Study in Mice. Pathology Working Group. Peer Review of Proliferative Vascular Lesions in Male and Female Mice. Report Identification CTL Study No. PM0937. EPL Project Number. 698-001. MRID 45710802. Unpublished.

A. Experimental Design

In this mouse oncogenicity study (MRID 44074201), poly(hexamethylenebiguanide) hydrochloride (PHMB, 20.2% a.i.) was administered in the diet to C57B1/10J$_f$CD-1/Alpk mice (55/sex/group) at 0, 400, 1,200 or 4,000 ppm (equivalent to 55, 167, or 715 mg/kg/day for males and 69, 217, or 856 mg/kg/day for females) for 2 years. **A Pathology Working Group (PWG) peer review re-evaluated the vascular lesions of this study in a report dated June 27, 2002 (MRID 45710802). Only the PWG re-read diagnoses of the vascular tumors are presented in this document.**

B. Discussion of Tumor Data

Survival Analysis

The statistical evaluation of mortality indicated a significant increasing trend with increasing doses of PHMB in female C57B1/10J$_f$CD-1/Alpk mice. There was no statistically significant incremental change in mortality with increasing doses of PHMB in male C57B1/10J$_f$CD-1/Alpk mice (Memo, L. Brunsman, 12/03/02, TXR No. 0051368). Tables 7 and 8 show the mortality results.

Table 7. PHMB - 1996 C57B1/10J$_f$CD-1/Alpk Mouse Study

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-52	53-78	79-105[f]	Total
0	0/55 (0)	0/55 (0)	5/55 (9)	24/50 (48)	29/55 (53)
400	0/55 (0)	2/55 (4)	6/53 (11)	18/47 (38)	26/55 (47)
1200	0/55 (0)	0/55 (0)	6/55 (11)	11/49 (22)	17/55 (31)*N
4000	0/55 (0)	5/55 (9)	7/50 (14)	17/43 (40)	29/55 (53)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[f]Final sacrifice at week 105.

( )Percent.

N: Negative change from control.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 8. PHMB - 1996 C57B1/10J$_f$CD-1/Alpk Mouse Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-52	53-78	79-105[f]	Total
0	1/55 (2)	0/54 (0)	7/54 (13)	17/47 (36)	25/55 (45)**
400	1/55 (2)	1/54 (2)	0/53 (0)	17/53 (32)	19/55 (35)
1200	1/55 (2)	0/54 (0)	5/54 (9)	14/49 (29)	20/55 (36)
4000	3/55 (5)	3/52 (6)	10/49 (20)	23/39 (59)	39/55 (71)**

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[f]Final sacrifice at week 105.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Tumor Analysis

The study pathologist and study peer reviewer determined that there was an increase in vascular tumors, mainly hemangiosarcoma, in both sexes at the high dose (4000 ppm) when compared to controls. The reviewing pathologist agreed with the increase in vascular tumors, but not always with the diagnosis (hemangioma vs. hemangiosarcoma). A pathologist working group (PWG) was conducted in 2002 to confirm the diagnoses of the vascular neoplasms (MRID 45710802). The PWG confirmed the study pathologist and peer reviewer's conclusion, that there was clear evidence of a treatment-related increase in incidence of animals with either hemangioma or hemangiosarcoma in the high dose of both sexes. This increase was largely due to the increase in the number of vascular tumors in the liver. PWG suggested that it is more appropriate to consider the total number of animals with vascular neoplasms, rather than the individual organs with either primary or metastatic lesions. Dr. J.M. Pletcher, EPA's consulting pathologist, confirmed the validity of the PWG report (TXR No. 0052033).

As shown in Table 9, male mice had significant increasing trends at $p<0.01$, and significant differences in the pair-wise comparisons of the 4000 ppm dose group with the controls, for hemangiomas ($p<0.01$), hemangiosarcomas ($p<0.05$) and combined hemangiomas and/or hemangiosarcomas ($p< 0.01$). There was also a significant increasing trend at $p < 0.01$, and a significant difference in the pair-wise comparison of the 4000 ppm dose group with the controls at $p < 0.05$, for rectal-anal junction squamous cell carcinomas.

Female mice had significant increasing trends for hemangiomas ($p<0.05$), hemangiosarcomas ($p<0.05$), and combined hemangiomas and hemangiosarcomas ($p<0.01$). There was also a significant difference in the pair-wise comparison of the 4000 ppm dose group with the controls, for combined hemangiomas and/or hemangiosarcomas, at $p< 0.05$. There was also a significant increasing trend at $p< 0.01$, and a significant difference in the pair-wise comparison of the 4000 ppm dose group with the controls at $p< 0.01$ for rectal-anal junction squamous cell carcinomas.

The statistical analyses of the male mice were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. The statistical analyses of the female mice were based upon Peto's prevalence test (Memo, L. Brunsman, 12/03/02, TXR No. 0051368). See Tables 9 through 12 for tumor analyses results.

Historical control data regarding hemangiosarcomas from studies using the C57BL/10J/CD-1 Alpk mouse and conducted by the Syngenta Central Toxicology Laboratory (Alderley Park, UK) are provided (MRID 457108-04). The total number of rats with hemangiosarcoma recorded, irrespective of site are summarized in Table 13. There are no historical control data for hemangiosarcoma by tissue.

Table 9. PHMB - 1996 C57B1/10J$_f$CD-1/Alpk Mouse Study

2002 PWG Re-Read

Male Vascular Tumor Rates[+] (All Sites) and Fisher's Exact Test and Exact Trend Test Results (p values)

	Dose (ppm)			
	0	400	1200	4000
Hemangiomas (%)	2/55 (4)	3/55 (5)	4/55 (7)	11[a]/53 (21)
p =	0.0007**	0.5000	0.3393	0.0063**
Hemangio-sarcomas (%)	5/55 (9)	4/55 (7)	6[b]/55 (11)	12/53 (23)
p =	0.0067**	0.5000	0.5000	0.0468*
Combined (%)	6[c]/55 (11)	6[c]/55 (11)	9[c]/55 (16)	20[d]/53 (38)
p =	0.0000**	0.6195	0.2899	0.0010**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 39.

[a]First hemangioma observed at week 39, dose 4000 ppm.
[b]First hemangiosarcoma observed at week 59, dose 1200 ppm.
[c]One animal in each of the 0, 400 and 1200 ppm dose groups had both an hemangioma and an hemangiosarcoma.
[d]Three animals in the 4000 ppm dose group had both an hemangioma and an hemangiosarcoma

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 10. PHMB - 1996 C57B1/10J$_f$CD-1/Alpk Mouse Study

Male Rectal-Anal Junction Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results (p values)

	Dose (ppm)			
	0	400	1200	4000
Squamous Cell Carcinomas (%)	0/45 (0)	0/45 (0)	0/45 (0)	5[a]/48 (10)
p =	0.0011**	1.0000	1.0000	0.0329*

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 36.

[a]First squamous cell carcinoma observed at week 36, dose 4000 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 11. PHMB - 1996 C57B1/10J$_f$CD-1/Alpk Mouse Study

2002 PWG Re-Read

Female Vascular Tumor Rates[+] (All Sites) and Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	400	1200	4000
Hemangiomas	6/51	2/53	5[a]/52	8/44
(%)	(12)	(4)	(10)	(18)
p =	0.0294*	-	-	0.1884
Hemangio-sarcomas	6/54	4/53	4/54	10[b]/49
(%)	(11)	(8)	(7)	(20)
p =	0.0300*	-	-	0.1262
Combined	8[c]/54	5[d]/53	7[e]/54	15[f]/49
(%)	(15)	(9)	(13)	(31)
p =	0.0023**	-	-	0.0288*

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First hemangioma observed at week 69, dose 1200 ppm.
[b]First hemangiosarcoma observed at week 54, dose 4000 ppm.
[c]Four animals in the 0 ppm dose group had both an hemangioma and an hemangiosarcoma.
[d]One animal in the 400 ppm dose group had both an hemangioma and an hemangiosarcoma.
[e]Two animals in the 1200 ppm dose group had both an hemangioma and an hemangiosarcoma.
[f]Three animals in the 4000 ppm dose group had both an hemangioma and an hemangiosarcoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.0$

Table 12. PHMB - 1996 C57B1/10J$_f$CD-1/Alpk Mouse Study

Female Rectal-Anal Junction Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	400	1200	4000
Squamous Cell Carcinomas (%)	0/42 (0)	0/42 (0)	0/43 (0)	8[a]/28 (29)
p =	0.0000**	-	-	0.0002**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First squamous cell carcinoma observed at week 86, dose 4000 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 13. Historical control data regarding hemangiosarcomas from studies using the C57BL/10J/CD-1 Alpk mouse and conducted by the Syngenta Central Toxicology Laboratory (Alderley Park, UK).

TOTAL NUMBER OF MICE WITH HEMANGIOSARCOMA RECORDED, IRRESPECTIVE OF SITE			
Study	Start Date	Male	Female
Study 1	1985/02	15/100 (15%)	0/100 (0%)
Study 2	1985/03	9/100 (9%)	4/100 (4%)
Study 3	1985/04	7/100 (7%)	4/100 (4%)
Study 4	1986/11	8/100 (8%)	5/100 (5%)
Study 5	1988/03	4/60 (6.7%)	4/60 (6.7%)
Study 6	1988/06	6/60 (10%)	0/60 (0%)
Study 7	1989/03	3/60 (5%)	0/60 (0%)
Study 8	1990/04	11/60 (18.3%)	3/60 (5%)
Study 9	1990/08	1/55 (1.8%)	4/55 (7.3%)
Study 10	1991/09	8/55 (14.6%)	4/55 (7.3%)
Study 11	1992/05	5/55 (9.1%)	4/55 (7.3%)
Study 12	1992/10	5/55 (9.1%)	6/55 (10.9%)
Study 13	1994/11	3/55 (5.5%)	1/55 (1.8%)

C. Non-Neoplastic Lesions

At the 1,200 ppm level, mice were observed with decreased overall body weight gains (males ↓7%; females ↓2%); increases in hematology parameters ($p<0.05$) including hemoglobin in females (↑6%), hematocrit in females (↑6%), and RBCs in females (↑5%); decreased absolute weight of the liver in males (↓15%) and females (↓21%); increased incidences of gross pathological changes including distended caeca in females (4% treated vs. 0% in controls); traumatized pinnae of ears in males (36% treated vs. 4% controls) and females (44% treated vs. 4% controls); increased incidences of non-neoplastic lesions including luminal dilatation of the gall bladder in females (9% treated vs. 2% controls), hepatocyte hypertrophy of the liver in males (13% treated vs. 0% controls) and females (35% treated vs. 0% controls), increased ploidy of the liver in males (13% treated vs. 0% controls) and females (36% treated vs. 0% controls), pigmentation of the liver in females (11% treated vs. 0% controls), and inflammation of the rectal-anal junction in males (44% treated vs. 2% controls) and females (47% treated vs. 21% controls).

At the 4,000 ppm dose level, decreased overall body weight gain was observed in males (↓50%) and females (↓32%); increased food consumption ($p<0.05$ or 0.01) from approximately week 12 through termination in males (↑7-29%) and females (↑7-26%); decreased food utilization ($p<0.01$) during weeks 1-12 in males (↓40%) and females (↓20%); increased hemoglobin in males (↑7%) and females (↑17%), hematocrit in males (↑5%) and females (↑16%), and RBCs in males (↑10%) and females (↑17%); decreased weight of the liver in males (20%) and females (30%); decreased weight of the testes of males (↓15%) and in adrenals of females (↓22%); and increased incidences of gross pathological changes including swollen anuses in males (18% treated vs. 0% controls) and females (7% treated vs. 0% controls), distended caeca in males (9% treated vs. 0% controls) and females (13% treated vs. 0% controls), pinnae of ears traumatized in males (27% treated vs. 4% controls) and females (22% treated vs. 4% controls), distention of the gall bladder in males (49% treated vs. 11% controls) and females (47% treated vs. 9% controls), and liver mass in males (23% treated vs. 9% controls) and females (15% treated vs. 8% controls). Increased incidences of non-neoplastic microscopic lesions were observed, including luminal dilatation of the gall bladder in males (48% treated vs. 20% controls) and females (39% treated vs. 2% controls), epithelial hyperplasia of the gall bladder in males (25% treated vs. 0% controls) and females (13% treated vs. 0% controls), hepatocyte hypertrophy of the liver in males (53% treated vs. 0% controls) and females (49% treated vs. 0% controls), ploidy of the liver in males (53% treated vs. 0% controls) and females (38% treated vs. 0% controls), pigmentation of the liver in males (36% treated vs. 0% controls) and females (42% treated vs. 0% controls), and inflammation of the rectal-anal junction in males (82% treated vs. 2% controls) and females (74% treated vs. 21% controls).

Toxicity observed common to both sexes of the 1,200 and 4,000 ppm treatment groups included decreased overall weight gains, pinnae of ears traumatized, hepatocyte hypertrophy of the liver, increased ploidy of the liver, and inflammation of the rectal-anal junction. There was also epithelial hyperplasia of the gallbladder common to females of both treatment groups, but occurring in males only at 4,000 ppm. No treatment-related effects were seen in mice in the 400 ppm dose group.

D. Adequacy of Dosing for Assessment of Carcinogenicity

Dosing was considered to be excessive at the high dose of 4000 ppm based on decreased overall body weight gains of 50% in males and 32% in females at termination. In addition, decreased body weight gains of 33% in males and 19% in females were observed at 13 weeks. The study indicated that the animals at the 4000 ppm dose group had increased food consumption ($p<0.05$ or 0.01) from approximately week 12 through termination in males (↑7-29%) and females (↑7-26%).

Dosing was considered to be adequate, but not excessive, in both males and females at the mid-dose of 1200 ppm based on decreased overall body weight gains (males, 7%; females, 2%), and increases in hematology parameters, and non-neoplastic histopathological changes in the gall bladder, liver, and rectal-anal junction.

3. Dermal Carcinogenicity Study in Mice (1977)

Reference:

Clapp, MJ.L.; Iswarn, T.J.; Major, P. (1977). Polyhexamethylene Biguanide:80 Week Skin Painting Study in Mice: Report No. CTL/P/331 (Amended). ICI Americas, Inc., Washington, Del; CDL: 233269. MRID 00066475. Unpublished Data.

ICI Americas, Inc. (1975). Polyhexamethylene Biguanide: 80 Week Painting Study in Mice: Appendix B: Pathology Individual Animal Data: Report No. CTL/P/331. CDL:235604-A. MRID 00104796.

A. Experimental Design

Four groups of specific pathogen free (50M + 50F) Alderley Park Mice received dermally 0.3 mL of test material at doses of 0 (solvent in ethanol), 0.6 mg (0.2% PHMB in ethanol), 6.0 mg (2% PHMB in ethanol) and 30.0 mg (10% PHMB in ethanol) per day for five days a week for 80 weeks. The treatment dosages are approximately equivalent to 0, 15, 150 and 750 mg/kg/day of a 20% PHMB solution.

B. Discussion of Tumor Data

Survival Analysis

The statistical evaluation of mortality indicated a significant increasing trend with increasing doses of PHMB in male and female SPF Alderley Park mice (Memo, L. Brunsman, 12/03/02, TXR No. 0051368). Tables 14 and 15 show the mortality results.

Tumor Analysis

There were no compound-related tumors observed in male mice. Female mice had significant increasing trends in liver angiosarcomas ($p < 0.01$) and vascular tumors from all sites combined ($p < 0.05$). There was a significant difference in the pair-wise comparison of the 30 mg dose group with the controls for liver angiosarcomas at $p < 0.05$. The statistical analyses of the female mice were based upon Peto's prevalence test. See Table 16 for tumor analyses results.

Table 14. PHMB - 1977 SPF Alderley Park Mouse Study

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (mg/animal) (mg/kg/day of 20% PHMB equivalent)	Weeks 1-20	21-40	41-60	61-81[f]	Total
0.0 (0.0)	0/49# (0)	1/49 (2)	6/48 (12)	9/42 (21)	16/49 (33)**
0.6 (15)	1/50 (2)	0/49 (0)	1/49 (2)	7/48 (15)	9/50 (18)
6.0 (150)	1/50 (2)	0/49 (0)	6/49 (12)	6/43 (14)	13/50 (26)
30.0 (750)	3/50 (6)	4/47 (9)	7/43 (16)	25/36 (69)	39/50 (78)**

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[f]Final sacrifice at week 80.

#: One animal missexed and removed from study at week 4.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 15. PHMB - 1977 SPF Alderley Park Mouse Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (mg/animal) (mg/kg/day of 20% PHMB equivalent)	Weeks 1-20	21-40	41-60	61-81[f]	Total
0.0 (0.0)	2/50 (4)	2/48 (4)	4/46 (9)	6/42 (14)	14/50 (28)**
0.6 (15)	1/50 (2)	1/49 (2)	3/48 (6)	11/45 (24)	16/50 (32)
6.0 (150)	1/50 (2)	2/49 (4)	7/47 (15)	8/40 (20)	18/50 (36)
30.0 (750)	3/50 (6)	2/47 (4)	7/45 (16)	27/38 (71)	39/50 (78)**

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[f]Final sacrifice at week 80.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 16. PHMB - 1977 SPF Alderley Park Mouse Study

Female Vascular Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose - mg/animal (mg/kg/day of 20% PHMB equivalent)			
	0	0.6(15)	6.0(150)	30.0(750)
Liver Hemangio-endotheliomas (%)	0/37 (0)	0/40 (0)	0/37 (0)	1[a]/31 (3)
p =	0.1059	-	-	-
Liver Angiosarcomas (%)	0/41 (0)	0/45 (0)	0/40 (0)	2[b]/35 (6)
p =	0.0025**	-	-	0.0401*
Other Sites (NOT LIVER) Hemangio-endotheliomas (%)	1[c]/41 (2)	0/44 (0)	0/40 (0)	0/33 (0)
p =	-	-	-	-
Combined (%)	1/41 (2)	0/45 (0)	0/40 (0)	3/35 (9)
p =	0.0129*	-	-	0.1606

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First liver hemangioendothelioma observed at week 68, dose 30.0 mg/kg/day.

[b]First liver angiosarcoma observed at week 60, dose 30.0 mg/kg/day.

[c]First other sites (NOT LIVER) hemangioendothelioma observed at week 62, dose 0.0 mg/kg/day.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Historical control data regarding hemangiosarcomas from studies using the Alderley Park mice and conducted by the ICI Central Toxicology Laboratory are provided (MRID 225025201) and are summarized in Table 17.

Table 17. Historical Control Data Regarding the Incidence of Liver Tumors in Control Alderley Park Mice in 80 Week Studies at the ICI Central Laboratory (MRID 225025201)

	1		2		3		4		5		6	
	1965		1065		1969		1969		1970		1972	
	M	F	M	F	M	F	M	F	M	F	M	F
Hepatosarcoma	4	1	3	0	1	0	3	0	0	0	0	0
Hepatoma	2	0	5	0	5	0	9	4	5	1	4	0
Hemangioendothelioma	0	0	0	0	0	1	0	0	0	0	0	0
Total no. of tumors	6	1	8	0	6	0	12	4	5	1	4	0
No. of animal examined	41	39	35	39	30	31	45	50	32	39	45	49
% each sex with tumors	14.6	2.6	22.9	0	20	0	26.7	8	15.6	2.6	8.9	0
Combined % with tumors	8.75		12.5		9.8		16.8		8.6		4.3	

Overall Incidence of Liver Tumors = 9.9%

C. Non-Neoplastic Lesions

The treatment dosages are equivalent to 0, 15, 150 and 750 mg/kg/day of 20% PHMB solutions. Mice that received the highest dose level showed a poorer condition, being very thin throughout the experiment. Death in both males and females in the highest dose group were slightly higher than in other groups during the first year. This pattern continued throughout the remainder of the study, resulting in a high mortality rate (75% in males and females) in the highest dose animals at termination, compared with approximately 30% in the other groups. The highest dose level of PHMB resulted in noticeable irritation to the skin of both males and females immediately after application. Erythema and some clumping of the growing fur were noticed during the first few weeks and after the 4th week; hyperkeratosis became evident, especially in males. No differences were apparent between the controls and those mice receiving 0, 0.6 or 6.0 mg PHMB per mouse per application. A significant reduction in mean body weight was observed for both male and female animals that received the highest dose level. There were no overall differences in food consumption between the control and treatment groups.

D. Adequacy of Dosing for Assessment of Carcinogenicity

Dosing was considered to be excessive at the high dose (750 mg/kg/day of 20% PHMB equivalent) in both sexes due to increased mortality (78%, high dose, versus 33% and 28% in the male and female controls, respectively) and decreased body weight gain. Overall body weight gain decreases of 45% and 17% were seen in males and females, respectively. No treatment-related effects were noted at the mid-dose level of 150 mg/kg/day of 20% PHMB equivalent (NOAEL).

IV. TOXICOLOGY

1. Metabolism

Bioavailability of PHMB was investigated in male and female Sprague-Dawley rats, fed diets of 200ppm or 2000ppm (10 and 100 mg/kg nominal dose) for fourteen days followed by a single radiolabelled dose of either 0.08 mg/kg or 0.8 mg/kg (MRID #'s 43567001 and 43599901), or after a single 100 mg/kg dose (MRID #'s 00077926 and 00086363). In both studies, feces represented the major route of excretion at all dose levels, comprising greater than 90% of the administered dose. A similar excretion in feces was observed in bile-cannulated rats after a single radiolabelled dose of 20 mg/kg (MRID # 43567001). Thus, fecal excretion of PHMB-derived radioactivity represents unabsorbed test material. The excretion pattern of low, mid, and high molecular weight fractions of PHMB was similar. Bioavailability was 4.7% and 3.9% for males and females, respectively, at the 10 mg/kg dose, and 3.0% and 2.6% for males and females, respectively, at the 100 mg/kg dose. Tissue distribution in rats given 10 mg/kg PHMB showed concentrations in the liver and kidney of male rats to be 0.568 g/g and 0.499 g/g, respectively. In female rats, liver and kidney concentrations were 0.752 g/g and 0.807 g/g respectively. As a percentage of the dose, liver of male and female rats contained 0.18% and 0.19% of the dose respectively, while kidneys contained 0.03% and 0.04% of the dose respectively. Metabolite analysis of pooled urine from rats administered a low molecular

weight fraction of PHMB at 20 mg/kg (the fraction showing the greatest absorption) revealed the presence of more than one metabolite, but identification was not performed due to the small amount of sample available for analysis.

2. Mutagenicity

In a battery of mutagenicity assays, PHMB was not mutagenic in bacteria or clastogenic in cultured human lymphocytes. There was also no evidence of in vivo clastogenicity or aneugenicity in mouse bone marrow or unscheduled DNA synthesis in rat liver. No mutagenic studies were found in the open literature. The following guideline studies were acceptable and satisfy the pre-1991 requirements for mutagenicity testing.

In two independently performed microbial gene mutation assays (MRID No. 41687004), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 were exposed to 0.32 - 500 μg/plate Vantocil IB (19.6% a.i.) in the absence or presence of S9 activation. Additional testing was carried out using comparable doses with and without S9 in TA1537 and TA98. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide. The utility of testing a bactericidal agent in a microbial mutation assay is questionable. Nevertheless, cytotoxicity was observed for the majority of strains at $\geq$ 200 µg/plate +/- S9. All strains responded in the expected manner to the nonactivated and S9-activated positive controls. There was, however, no evidence that Ventocil IB induced a mutagenic response in any strain at any nonactivated or S9-activated dose.

In an in vitro mammalian cell cytogenetic assay (MRID No. 41404501/42149905), human lymphocytes derived from male and female donors were exposed to Vantocil IB (19.6% a.i. in water) doses of 5, 25, or 50 μg/mL without S9 activation (both donors) and levels of 25, 100 or 187.5 μg/mL + S9 (male donor) or at 25, 100 or 250 μg/mL (female donor) with S9 activation for approximately 2.5 - 3.5 hours. The S9 liver homogenate was derived from Aroclor 1254 induced Sprague-Dawley rat livers. The test material was delivered to the test system in physiological saline. A 50% reduction in mitotic index occurred at 50 μg/mL - S9 (both donors) and at 100 μg/mL + S0 (male donor) or at 250 μg/mL + S9 (female donor). The positive controls induced the expected high yield of chromosome aberrations in the lymphocytes derived from the male and female donors. There was, however, no evidence that Vantocil IB induced a clastogenic effect. This study is classified as Acceptable and satisfies the guideline requirement for an in vitro cytogenetic assay.

In a mouse micronucleus assay (MRID No. 41096901/41404503), groups of five male and five female C57BL/6JfCD-1/Alpk mice received single oral gavage administrations of 250 or 400 mg/kg Vantocil IB (19.6% a.i.) prepared in deionized water. Bone marrow cells from mice in the high-dose group were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs). Low-dose animals were sacrificed at 24 hours. Two animals receiving 400 mg/kg died prior to the scheduled sacrifice. There was also clear evidence of target cell cytotoxicity in the high-dose males and females at all sacrifice intervals. The positive control induced the expected high yield of MPEs in males and females. Vantocil IB did not, however, induce a clastogenic or aneugenic effect in

either sex at any dose or sacrifice time. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a micronucleus assay.

In two independently performed in vivo/in vitro unscheduled DNA synthesis (UDS) assays (MRID No. 41404502/42149903), groups of two to three male rats were administered single oral gavage doses of 750 or 1500 mg/kg Vantocil IB (19.6%) prepared in deionized water. Animals were sacrificed at 4 and 12 hours post treatment and recovered hepatocytes were scored for UDS. Clinical toxicity (i.e., excessive salivation and subdued nature) was observed at 1500 mg/kg; higher levels were lethal. No cytotoxicity for the target organ was seen at either level. The positive control induced the expected high yield of hepatocytes with net nuclear grains. There was, however, no evidence that the Vantocil IB induced a genotoxic response at either dose or sacrifice time. This study is classified as Acceptable and satisfies the guideline requirement for a UDS assay (84-4).

3. Structure-Activity Relationship

No appropriate structural analogs could be located for comparison purposes. In general, with the exception of exposure via the inhalation route, high MW polymers are not expected to be of significant carcinogenic concern because of poor bioavailability. PHMB has indeed been shown to have low bioavailability. However, SAR consideration suggests that there is a likelihood that the lower MW fractions of PHMB may be metabolized to polyamines in analogy to the metabolism of arginine (a guanidine-containing amino acid) to form biogenic polyamines such as putrescine, spermidine and spermine. There is strong evidence that these polyamines are readily taken up by cells and may play an important role in promoting skin and colon carcinogenesis by serving as ingredients to support neoplastic growth. The metabolism study submitted by the company showed that low MW fractions of PHMB can indeed be metabolized in the rat; however, the metabolites have not yet been identified. It would be important to carry out the identification study. If the metabolites can be identified to be polyamines, they could provide a potential mode of action to explain the carcinogenicity of PHMB.

4. Subchronic and Chronic Toxicity

a) Subchronic Toxicity

Rats

In a 90-day rat oral toxicity study, young adult specific pathogen free (S.P.F) Wister rats (25M + 25 F) received levels of 0, 2500, and 5000 ppm (0, 310, 620 mg/kg/day) of poly(hexamethylenebiguanide) hydrochloride (25%) for 90 days in the diet. At the end of the 90-day test period all animals were killed with chloroform, and an immediate postmortem examination was made. All animals survived the 90-day test period. There were no specific adverse effects of the compound. Food consumption was comparable for the test groups and controls. Body weight was moderately reduced in males (13.2% less than controls), and females (16.6% less than controls) fed the compounds at the highest dietary level (5000 ppm). No abnormalities in hematological parameters were observed. No gross abnormalities were observed. No remarkable change in organ/body weight ratios was detected. Microscopic evaluation revealed that the liver of some females given the compound at a level of 5000 ppm showed an unusual degree of iron pigment both within the liver cells and kupffer cells. Although the report states that no iron pigments were seen in animals fed 2500 ppm test material in the diet, the study does not include detailed histopathological results of the 2500 ppm animals. Therefore, the NOAEL cannot be determined in this study.

Dogs

In a 90-day dog oral toxicity study, three groups of Beagle dogs (4M + 4F per group), 12.4 - 14.6 kg initial body weight, received poly(hexamethylenebiguanide) hydrochloride (25%) at dietary levels of 0, 5500 and 11,000 ppm (0, 137 or 275 mg/kg/day) ad libitum for 90 days. General observation, food consumption, body weight and the following clinico-pathological studies were performed: hemoglobin, packed cell volume (hematocrit), leucocyte count (total), leucocyte count (differential), blood urea, serum alkaline phosphatase, BSP (liver function test) and urine analyses (pH, specific gravity, glucose, protein, bilirubin, and microscopy of centrifuge deposits). At the end of the test period the animals were killed with an overdose of pentobarbitone administered intravenously. A full postmortem examination and microscopic examination were taken from the following: brain (cerebrum, cerebellum and pons), spinal cord, pituitary, submaxillary gland, thyroid, thymus, heart, lung, aorta, stomach, duodenum, ileum, colon, liver, spleen, kidney, bladder, adrenal, ovary and uterus or testis and epididymis, and sciatic nerves.

Both treated and control animals maintained an excellent condition throughout and no adverse effects were noted. No food consumption data was submitted. Mean body weights were comparable between control and treated animals except for the 11,000 ppm female dogs which gained significantly less total weight than the control female dogs. Results of the hematological parameters were unremarkable. Clinical function tests showed no difference in retention of BSP attributable to test material. Urine analyses did not appear to be influenced by treatment.

Organ/body weight ratios showed no significant variation from the normal as a result of treatment. No gross pathology attributable to test material was detected. Microscopic examination revealed slight hemosiderons in 2 out of 4 males at 11,000 ppm. No other microscopic abnormalities attributable to treatment were present at either dose level.

The NOAEL for this 90-day dog feeding study appears to be 5500 ppm (low dose). The high dose treatment caused less total weight gain in female dogs and slight hemosiderons in 2 out of 4 male dogs.

b) Chronic Toxicity

Dogs

In a chronic toxicity study (MRID # 43620501), poly(hexamethylenebiguanide) hydrochloride (25%) 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%, 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 treatment. Marked dermatitis of the limbs was also observed in this dog. The LOAEL 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 clinical signs of toxicity and clinical chemistry alterations in the female dog. The NOAEL 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.

5. Mode of Action Studies

There are no mode of action studies available at this time.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

The CARC concluded that PHMB showed evidence of carcinogenicity based on the following:

Rat - Oral

There was no treatment-related increase in any tumors in male Wistar rats.

Vascular Tumors: Female Wistar rats from the PWG diagnoses had significant increasing trends in liver hemangiomas and liver hemangiosarcomas, both at $p < 0.05$. There was also a significant increasing trend for hemangiomas and/or hemangiosarcomas at all sites combined, at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 2000 ppm dose group with the controls for liver hemangiomas and hemangiomas and/or hemangiosarcomas at all sites combined, both at $p < 0.05$. The incidence of hemangiomas and hemangiosarcomas for all sites combined was 2/42 (5%), 1/42 (2%), 3/40 (8%), and 6/35 (17%) for the 0, 200, 600, and 2000 ppm dose groups, respectively. Historical control data regarding hemangiosarcomas shows the range of hemangiosarcomas by tissue in rats was 0-1.9%. **The CARC considered the tumor response at the high dose (17% for the combined re-read of liver and original diagnoses at other sites) to be treatment-related and driven by the increase in hemangiomas when all sites are combined.**

Adequacy of Dosing: The CARC concluded that dosing at the highest dose (2000 ppm) was considered to be adequate, but not excessive, for both male and female rats. In high dose females, this was based on increased mortality and a reduction in body weight of 5-8% throughout the study. In high-dose males, body weights were significantly reduced (4-6%) through week 79. Food utilization (g growth/100 g feed) for the first 12 weeks decreased significantly (↓7-8%) in both sexes at 2000 ppm.

Mouse - Oral

Vascular Tumors: Male C57B1/10J$_f$CD-1/Alpk mice from the PWG diagnoses had significant increasing trends in hemangiomas, hemangiosarcomas, and combined hemangiomas and hemangiosarcomas, all at $p<0.01$. There were also significant differences in the pair-wise comparisons of the 4000 ppm dose group with the controls, for hemangiomas ($p<0.01$), hemangiosarcomas ($p<0.05$), and combined hemangiomas and/or hemangiosarcomas ($p< 0.01$). The incidence of hemangiomas was 2/55, 3/55, 4/55, 11/53, for the 0, 400, 1200, and 4000 ppm dose groups, respectively. The incidence of hemangiosarcomas was 5/55, 4/55, 6/55, and 12/53 for the 0, 400, 1200, and 4000 ppm dose groups, respectively. The incidence of combined hemagiomas and hemangiosarcomas was 6/55, 6/55, 9/55, and 20/53 for the 0, 400, 1200, and 4000 ppm dose groups, respectively. For hemangiosarcomas, the tumor incidence in the 4000 ppm dose group (23%) exceeded the historical control range (1.8-

18.3%). **The CARC considered the increase in vascular tumors (hemangiomas, hemangiosarcomas, and combined) at the high dose (which is considered to be excessive) to be treatment-related. Although not statistically significant, the tumor response (9/55 [16%] for the combined hemangiomas and hemangiosarcomas) in mice at the mid-dose of 1200 ppm is considered treatment-related since this tumor type was also seen in female mice (orally and dermally) and female rats.**

Vascular Tumors: Female C57B1/10J$_f$CD-1/Alpk mice from the PWG diagnoses had significant increasing trends in hemangiomas ($p<0.05$), hemangiosarcomas ($p<0.05$), and combined hemangiomas and hemangiosarcomas ($p<0.01$). There was also a significant difference ($p< 0.05$) in the pair-wise comparison of the 4000 ppm dose group with the controls, for combined hemangiomas and/or hemangiosarcomas. The incidence of combined hemangiomas and/or hemangiosarcomas was 8/54 (15%), 5/53 (9%), 7/54 (13%), and 15/49 (31%) for the 0, 400, 1200, and 4000 ppm dose groups, respectively. For hemangiosarcomas, the tumor incidence in the 4000 ppm dose group (31%) exceeded the historical control range (0-10.9%). There are no historical control data for hemangiomas by tissue. **The CARC considered the combined vascular tumors at the high dose (which is considered to be excessive) to be treatment-related. There were no increases in these tumors at doses which were not considered excessive.**

Rectal-Anal Junction Tumors: In male mice there was a significant increasing trend at $p < 0.01$ and a significant difference in the pair-wise comparison of the 4000 ppm dose group with the controls at $p < 0.05$, for rectal-anal junction squamous cell carcinomas. The incidence of rectal-anal junction tumors was 0/45, 0/45, 0/45, and 5/48 for the 0, 400, 1200 and 4000 ppm dose groups, respectively. In female mice there was a significant increasing trend at $p< 0.01$, and a significant difference in the pair-wise comparison of the 4000 ppm dose group with the controls at $p< 0.01$, for rectal-anal junction squamous cell carcinomas. The incidence of rectal anal junction tumors was 0/42, 0/43, 0/43, 8/28 for the 0, 400, 1200 and 4000 ppm dose groups, respectively. **The CARC considered the carcinomas at the high dose (which is considered to be excessive) in both males and females to be treatment-related but suggestive of a local irritant effect. These tumors did not contribute to the CARC's weight-of-the-evidence analyses.**

Adequacy of Dosing

Dosing was considered to be excessive at the high dose of 4000 ppm based on decreased overall body weight gains of 50% in males and 32% in females at termination. In addition, decreased body weight gains of 33% in males and 19% in females were seen at 13 weeks. The study indicated that the animals at the 4000 ppm dose group had increased food

consumption ($p<0.05$ or 0.01) from approximately week 12 through termination in males (↑7-29%) and females (↑7-26%).

Dosing was considered to be adequate, but not excessive, in both males and females at the mid-dose of 1200 ppm based on decreased overall body weight gains (males, 7%; females, 2%), increases in hematology parameters, and non-neoplastic histopathological changes in the gall bladder, liver, and rectal-anal junction.

Mouse - Dermal

Vascular Tumors: Female Alderley Park mice had significant increasing trends in liver angiosarcomas ($p < 0.01$) and vascular tumors from all sites combined ($p < 0.05$). There was also a significant difference in the pair-wise comparison of the 30 mg dose group with the controls for liver angiosarcomas at $p < 0.05$. The incidence of liver angiosarcomas was 0/41, 0/45, 0/40, and 2/35 for the 0, 0.6, 6.0, and 30 mg dose groups, respectively. **The CARC considered the tumor increase at the high dose to be equivocal since the tumors were only seen at an excessive dose. Vascular tumors were not seen at lower doses.**

There was no treatment-related increase in any tumors in male Alderley Park mice.

Adequacy of Dosing

Dosing was considered to be excessive at the high dose (750 mg/kg/day of 20% PHMB equivalent) in both sexes due to increased mortality (78%, high dose, versus 33% and 28% in the male and female controls, respectively) and decreased body weight gain. Overall body weight gain decreases of 45% and 17% were seen in males and females, respectively. No treatment-related effects were noted at the mid-dose level of 150 mg/kg/day of 20% PHMB equivalent (NOAEL).

2. Mutagenicity

There is no mutagenicity concern.

3. Structure Activity Relationship

No appropriate structural analogs could be located for comparison purposes. In general, with the exception of exposure via the inhalation route, high molecular weight (MW) polymers are not expected to be of significant carcinogenic concern because of poor bioavailability. PHMB has indeed been shown to have low bioavailability. However, SAR consideration

suggests that there is a likelihood that the lower MW fractions of PHMB may be metabolized to polyamines in analogy to the metabolism of arginine (a guanidine-containing amino acid) to form biogenic polyamines such as putrescine, spermidine and spermine. There is strong evidence that these polyamines are readily taken up by cells and may play an important role in promoting skin and colon carcinogenesis by serving as ingredients to support neoplastic growth. A metabolism study submitted by the company showed that low MW fractions of PHMB can indeed be metabolized in the rat; however, the metabolites have not yet been identified.

4. Mode of Action

No mode of action studies were available.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA *Draft Guidelines for Carcinogen Risk Assessment* (July 1999), the CARC classified PHMB into the category **"Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential"** by the oral and dermal routes. The weight-of-the-evidence considerations for this classification are as follows:

(i) A treatment-related statistically significant increase (trend and pair-wise) in vascular tumors (mainly benign) was seen in female rats at an oral dose that was considered to be adequate, but not excessive. This was considered as the strongest evidence in the CARC's evaluation of PHMB.

(ii) Oral exposure to male and female mice also resulted in treatment-related vascular tumors seen at an excessive dose. However, at the next highest dose level, which was considered adequate but not excessive, there was a slight but not statistically significant increase in this same tumor which added to the CARC's concern for this tumor type.

(iii) It is noted that dermal exposure to female mice resulted in an equivocal increase in vascular tumors seen at only an excessive dose.

(iv) No treatment-related increase in any tumors was seen in male rats via the oral route or in male mice via the dermal route of exposure.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The quantification of human cancer risk is not required.

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