10-16-96

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Special Metabolism Studies with Polyhexamethylene Biguanide (PHMB).

EPA Identification Numbers:
DP Barcode: D213000; D214021 MRID #'s 43567001 and 43599901
P.C. Code: 111801 Submissions: S483318 and S485038

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

FROM: Timothy F. McMahon, Ph.D. 10/8/96
Pharmacologist, Review Section I
Toxicology Branch II, Health Effects Division (7509C)

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

and

Yiannakis M. Ioannou, Ph.D 10/16/96
Acting Chief, Toxicology Branch II
Health Effects Division (7509C)

Registrant: Zeneca Ag Products

Action Requested: Review of special metabolism studies conducted with PHMB in the rat.

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Recommendations: Toxicology Branch II has reviewed the special metabolism studies in the rat (MRID #'s 43567001 and 43599901) and has determined that the studies are **acceptable**, and, in conjunction with earlier studies (MRID #'s 00077926 and 00086363), that the §85-1 guideline [OPPTS 870.7485] is satisfied.
A single executive summary describing all three studies is presented below.

Executive Summary

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 µg/g and 0.499 µg/g, respectively. In female rats, liver and kidney concentrations were 0.752 µg/g and 0.807 µg/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.

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Reviewed by: Timothy F. McMahon, Ph.D. _____ Date: 10/8/96
Pharmacologist, Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Jess. C. Rowland, M. S. _____ Date: 10/10/96
Acting Head, Section I, Toxicology Branch II (H7509C)

Data Evaluation Record

Study type: Metabolism (Special Studies) P.C. Code: 111801

EPA identification numbers: EPA MRID numbers: 435999-01 and 435670-01
DP Barcode: D213000 and D214021
Submission: S483318 and S485038

Laboratory Project numbers: URO360 ; URO 468; CTL/P/163B

Test materials: [^{14}C]-Polyhexamethylene biguanide (PHMB); unlabelled PHMB; labelled and unlabelled low, mid, and high molecular weight fractions of PHMB

Synonyms: 2-chloro-2,6-diethyl-N-(methoxymethyl)acetanilide.

Testing Facilities: Zeneca Central Toxicology Laboratory, UK

Sponsor: Zeneca, Inc., Wilmington, Delaware

Title of reports:
[1]: Laboratory Project No. URO 468, " PHMB: Bioavailability Following Dietary Administration in the Rat."

[2]: Laboratory Project No.URO 360, " PHMB: Absorption, Distribution, Metabolism, and Excretion Following Single Oral Dosing (20 mg/kg) in the Rat."

[3]: Laboratory Project No. CTL/P/163B, "Absorption and Excretion Studies in the Rat."

Author(s): R.E. Lythgoe, E.F. Howard, E. Prescott

Reports issued: [1]:March 22, 1995; [2]: February 22, 1995; [3]: March, 1975.

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Executive Summary:

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 μg/g and 0.499 μg/g, respectively. In female rats, liver and kidney concentrations were 0.752 μg/g and 0.807 μg/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.

Classification: The studies when taken together are **acceptable**, and satisfy the §85-1 guideline [OPPTS 870.7485] requirement.

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I. MATERIALS

A. Test Material

Study 1

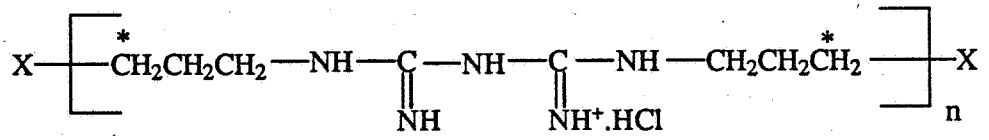
[1]: ^{14}C-PHMB
Radiochemical Purity: not stated; PHMB is a mixture of various molecular weight fractions
Specific Activity : 1.85 GBq/ 4mmol hexamethylene diamine

Unlabelled PHMB; 20% aqueous solution; mol. wt. 2650

[2]: ^{14}C-PHMB
Radiochemical Purity: not stated; PHMB is a mixture of various molecular weight fractions
Specific Activity : 1.85 GBq/ 4mmol hexamethylene diamine

In study #2, both unlabelled and labelled PHMB were fractionated into low, mid, and high molecular weight fractions. The mean weight average molecular weight for these fractions was: Low, molecular weight 1230-1233; Mid, 2070-3048; and High, 3800-4700.

Chemical Structure (* denotes position of radiolabel):



X = $-CH_2CH_2CH_2-NH_2^+.HCl$ or $-CH_2CH_2CH_2-NH-C(=NH)-NH.CN$

or $-CH_2CH_2CH_2-NH-C(=NH)-NH_2^+.HCl$

B. Vehicles: Sterile, double deionized water (study #2); In study #1, dietary preparation was used except for the oral radiolabelled dose, in which preparaed radiolabelled test diets (summarized below) were suspended in 1% gum tragacanth in sterile, double deionized water.

C. Test Animals: Species: rat, male and female
Strain: AlPk:APfSD
Source: BSS, Alderley Park, Alderley Edge, Macclesfield, Cheshire, UK.
Weights: study [1]: males, 280-338g ; females, 206-238g
study [2]: males, 171-270g; females, 163-250g.

II. METHODS

A. Study Design

In study #1, 8 male and 8 female rats were fed diets containing either 200 or 2000 ppm PHMB for fourteen days followed by a single oral radiolabelled dose. After the radiolabelled dose, rats were placed into glass metabolism cages (to which they had been previously acclimated) for collection of urine and feces at 24 hour intervals up to 3 days post-dose. After the last collection time period, rats were sacrificed under halothane anesthesia and the g.i. tract and contents removed for analysis of radioactivity. Residual carcass and plasma were also retained.

In study #2, three separate experiments were conducted. In the first, 3 male and 3 female bile duct-cannulated rats were given a single oral dose of 20 mg/kg radiolabelled PHMB. Urine, feces, and bile excretion of radioactivity were monitored for 2 days post-dose. In the second experiment, 3 groups of 4 male rats were given a single 20 mg/kg dose of either a low, mid, or high molecular weight fraction of radiolabelled PHMB. Urinary and fecal excretion of radioactivity were monitored for 3 days post-dose. In the third experiment, 5 male and five female rats were given a single 20 mg/kg dose of the low molecular weight fraction of PHMB, and urinary and fecal excretion of radioactivity measured. In addition, residual radioactivity in blood, selected tissues, and residual carcass was measured at sacrifice. Chromatographic analysis of urine samples was performed.

C. Experimental

1) Animal Husbandry

In study #1 and #2, rats were housed in groups of the same sex in stock rat cages and acclimated for either 7 days (study #1) or 3 days (study #2). During the acclimation period, rats were given free access to food (CT1 rat diet, Special Diet Dervices Ltd. in study #1; pelleted PCD rat diet, Special Diet Services Ltd., in study # 2) and water. In study #2, the rats given the oral doses of low, mid, or high molecular weigh fractions were starved for 11 hours pre-dose and for 3 hours post-dose to minimize binding of dosed material to diet present in the g.i. tract, as this would influence absorption. Acording to both reports, the

rats were housed under controlled environmental conditions in both studies (19-23 °C and 40-70% relative humidity in study #1; 17-23 °C and 14-76% relative humidity in study #2). The range of humidity in study #2 appears widely variable, but no explanation was given for this variability in study #2.

2) Dosing

In study #1, experimental diets containing either 200 or 2000 ppm unlabelled PHMB (the same doses used in the chronic feeding study in rats) were prepared by titrating the appropriate amounts of PHMB into 500 g batches of Laboratory Diet A of the CT1 diet. The premix was mixed in a Kenwood Chef Mixer for 10 minutes after which 2x250g of CT1 diet was added at 5 minute intervals to give a 1kg dry mix. This was then mixed with 14kg of CT1 diet and mixed in a Pharma Matrix blender for 4 minutes. The prepared diets were stored in 5kg batches at -20 °C and were allowed to thaw immediately prior to use. Radiolabelled diet was prepared in an analogous manner as the unlabelled diet. Radiolabelled diets were formulated into aqueous suspensions immediately prior to use. A stable suspension for oral dosing was prepared by mixing the required amount of radiolabelled diet with the 1% gum tragacanth solution and mixing by gentle shaking and milling.

According to the report, in study #1 rats given the low dietary concentration of PHMB received nominal doses of 0.08 mg/kg 14-C PHMB (0.2MBq/kg), while rats given the high dietary concentration of PHMB received nominal doses of 0.8 mg/kg 14-C PHMB (2MBq/kg). The amount of radiolabelled dose was determined by weighing the syringe and catheter assembly prior to and immediately after dosing. **Note:** It would have been more appropriate to determine the radioactivity of a dosing aliquot of the actual dose solutions rather than weighing the syringe.

In study #2, five dosing solutions were prepared for the three experiments conducted in this study. For the biliary excretion experiment, 46.1 mg of PHMB and 10.7MBq (equivalent to 4.2mg) of 14-C PHMB wewre mixed in the dose vehicle to give a final volume of 10ml and concentrations of 5.1 mg PHMB/g and 1.1MBq/g dosing solution. The specific activity of the test substance was 212kBq/mg of PHMB. For the administration of the low, mid, and high molecular weight fractions of PHMB, as well as for the excretion experiment, the following dose solution parameters were reported:

Dose Group	PHMB	14-C PHMB	final volume
low M.W. fraction	24.2mg	2.7MBq; 1.1mg	5ml
mid M.W. fraction	24.1mg	2.9MBq; 1.2mg	5ml
high M.W. fraction	48.0mg	5.4MBq; 2.1mg	10.0ml
excretion experiment	69.1mg	15.4MBq; 6.1mg	15.0ml

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Dose volume for the experiments in study #2 was stated as 4ml/kg. In study #1, it was noted that acheived concentration, homogeneity, and stablity were not performed for the dietary mixtures of PHMB. This issue has been discussed previously between the Agency and the registrant (HED document # 010753), and it was agreed that the data presented in this document would address the issues of concentration and homogeneity of PHMB in dietary mixtures. Thus, no analysis was necessary in the present submissions.

c. Sample Collection and Analysis

The collection of urine and feces in study #1 was mentioned above. Processing of collected samples was performed in replicate by liquid scintillation counting (urine and plasma), oxidation followed by liquid scintilation counting (dosing suspensions, feces, blood and tissues), or solubilization followed by liquid scintillation counting (carcass).

In study #2, bile and urine for the biliary excretion experiment were separately collected at 3 and 6 hours post-dose for bile, and at 12, 24, 36, and 48 hours post-dose for urine and feces. Bile was collected at room temperature, and urine and feces collected over solid carbon dioxide. In the bioavailability and excretion experiments, urine only was collected at 6 hours post-dose, and urine and feces collected separately at 12, 24, 36, 48, and 72 hours post-dose. Excreta were collected separately over solid carbon dioxide. Tissues were collected at either 48 hours post-dose (biliary experiment) or at 72 hours post-dose (bioavailability and excretion experiments).

For analysis of radioactivity, liquid scintillation counting (dosing solutions, urine, fecal extracts, bile, cage wash, plasma), oxidation followed by liquid scinillation counting (feces, blood and tissues) or solubilization followed by liquid scintillation counting (carcass, liver and abdominal fat) was employed. As in study #1, the amount of radiolabelled dose given to each rat was determined by weighing the syringe and catheter assembly prior to and immediately after dosing. The study report stated (page 24) that duplicate or triplicate samples of dosing solution were diluted volumetrically with water and aliquots of the dilutions taken for scintillation counting. The counting data for the dose solutions was not shown in the report. These data would have been a more accurate measure of the amount of radiolabel received by each rat.

d. Statistics

In study #1, the limit of detection was stated as 50 dpm per sample (twice the scintillation counter background rate), and for all tissues, 0.0008 μg equivalents PHMB per gram of tissue based on a sample size of 200 mg for all determinations. In study #2, the limit of detection for all tissues examined ranged between 0.0098-0.0198 μg equivalents PHMB per gram of tissue. For the purposes of group mean calculations, individual values which were below the limit of detection were accepted as being equal to the limit of detection.

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D. Compliance --

A signed statement of no data confidentiality claims was provided with both studies.

A signed statement of GLP compliance (40 CFR 160.35) was provided with both studies. In study #1, it was noted that the stability, homogeneity, and acheived concentration of the unlabelled test material in the diet could not be determined as required by GLP. This issue has been resolved previously between the registrant and the Agency.

A signed statement of quality assurance was provided with both studies.

A signed statement of EPA flagging criteria does not apply to these studies.

III. RESULTS

Study #1

The excretion of 14-C PHMB-derived radioactivity was summarized in Table 1, page 26 of MRID # 435999-01. At both the 200ppm and 2000ppm dose levels, fecal excretion of radioactivity was the sole significant route of excretion. For both dose levels, between 104-109% of the administered dose was excreted over 0-72 hours post-dose, with 90% or greater excreted in the first 24 hours post-dose at both dose levels. For the tissues analyzed for residual radioactivity at 72 hours post-dose (Table 2, page 27 of the report), less than 1% of the dose was observed in the g.i. tract and g.i. contents. For the carcass, between 1.1-1.2% of the dose was observed at the 200ppm dose, and between 0.22-0.25% of the dose was observed at the 2000ppm dose.

Based on the above data, the bioavailable dose can be considered as the amount of the dose excreted in urine together with that present in the carcass after removal of the g.i. tract and contents. Thus, the bioavailable dose at the low dose level can be considered as 4.7% and 3.9% for male and female rats, and 3.0% and 2.6% at the high dose level for male and female

rats, respectively. It is noted that at both dose levels, male rats absorb a higher percentage of the administered dose than female rats. In addition, less of an administered dose is absorbed by both sexes at the 2000ppm dose level than at the 200ppm dose level.

Study #2

A) Biliary Excretion Experiment

The results of bile cannulation experiments were summarized in Tables 1 and 2 of MRID # 435670-01, pages 43-44 of the report. These two tables summarized urinary, fecal, and biliary excretion of PHMB-derived radioactivity in male and female rats after a single 20 mg/kg radiolabelled dose. The total amount of the dose absorbed could be represented by the cumulative radioactivity excreted in urine and bile together with the remaining radioactivity in the carcass. As shown by the data in these tables, between 96.8%-98.9% of the administered dose was excreted in feces, with between 2.03%-2.67% excreted in urine. Biliary excretion represented between 0.12-0.14% of the administered dose in male and female rats. Thus, the fecal radioactivity was unabsorbed test chemical and was not excreted through the bile.

Tables 3 and 4, pages 45-46 of the report, summarized tissue and residual carcass measurements of radioactivity in male and female bile-duct cannulated rats given the single 20 mg/kg radiolabelled dose of PHMB. Blood levels at 48 hours post-dose were 0.02 μg equiv. / gram tissue for male rats, and 0.03 μg equiv. / gram tissue for female rats. The g.i. tract and contents of male rats contained 1.28% of the dose at 48 hours post-dose, while the g.i. tract and contents of female rats contained 2.51% of the dose at 48 hours post-dose. The residual carcass of male rats contained 0.27% of the dose, and the residual carcass of female rats contained 0.38% of the dose.
Taking these data into consideration, the absorption of PHMB (unfractionated) in male and female rats can be calculated as 2.42% of the dose and 3.19% of the dose. These values are in good agreement with the data presented in study #1 summarized above. However, in study #1, male rats showed a higher percentage of absorption.

B) Excretion of Low, Mid, and High Molecular Weight Fractions of PHMB

Table 5, page 47 of the report, summarized excretion of PHMB-derived radioactivity in male rats (4/group) which had received low, mid, and high molecular weight fractions of PHMB. The data showed the following excretion pattern for the low, mid, and high molecular weight fractions of PHMB:

	Low M.W. fraction	Mid M.W. fraction	High M.W. fraction
Urine (% of dose)	5.22 ± 0.66	0.15 ± 0.05	0.22 ± 0.03
Feces (% of dose)	94.94 ± 3.15	101.42 ± 2.48	96.03 ± 1.33

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As shown, the percentage of an administered dose excreted in urine was very low after administration of low, mid, or high molecular weight PHMB fractions, while the majority of a dose was excreted through the feces. However, it is noted that the low molecular weight fraction showed the highest percentage of excreted urinary radioactivity.

Tables 6 through 8 of the report showed tissue distribution of radioactivity for the g.i. tract, g.i. contents, blood , plasma, and residual carcass after administration of a low, mid, and high molecular weight fraction of PHMB. The percentage of a dose observed in the g.i. tract and contents was low, i.e. less than 0.3% of the dose for all fractions. It is noted that the percentage in the g.i. contents was higher than the percentage in the g.i. tract itself for all molecular weight fractions. Blood (0.10-0.15 μg equiv./g) and plasma ($<$0.02 μg equiv./g) levels of PHMB-derived radioactivity did not differ significantly among the three dose groups.

Tables 9 and 10 summarized excretion data from the definitive disposition experiment in which five male and five female rats were given a 20 mg/kg dose of 14-C PHMB low molecular weight fraction. This fraction was chosen as absorption was highest for the low molecular weight fraction. The results of this experiment can be summarized as follows:

Excretion of Low Molecular Weight Fraction PHMB in Male and Female Rats
(% Administered Dose)

	Males	Females
Urine	7.84 ± 3.03	2.55 ± 0.80
Feces	94.13 ± 1.94	93.46 ± 2.03
Cage Wash	0.33 ± 0.11	0.22 ± 0.13
Total	102.31 ± 2.53	96.23 ± 1.84

As with earlier experiments, the majority of the administered dose was excreted in feces of both male and female rats. Male rats showed a higher percentage excretion of low molecular weight fraction PHMB in urine than female rats. It is probable that urinary excretion of PHMB derived radioactivity consists solely of excretion of the low molecular weight fraction of PHMB, as urinary excretion is markedly reduced when the mid- and high-molecular weight fractions are administered.

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Tissue distribution of low molecular weight fraction PHMB-derived radioactivity was presented in Tables 11 and 12 for male and female rats, respectively. At 72 hours post-dose, the highest concentrations of radioactivity were observed in the liver and kidneys of male and female rats. In males, the concentrations in liver and kidney were 0.568 μg equivalents per gram and 0.499 μg equivalents per gram, respectively. In females, the concentrations in liver and kidney were 0.752 μg equivalents per gram and 0.807 μg equivalents per gram, respectively. Expressed as a percentage of the dose, the liver of male and female rats contained 0.18% and 0.19% of the dose, while the kidneys contained 0.03% and 0.04% of the dose in male and female rats respectively. In all other tissues examined, the concentration of radioactivity was much lower than that observed in liver and kidney (0.007-0.271 μg equivalents per gram).

2) Metabolite Characterization

Chromatographic analysis, using size-exclusion chromatography, was performed on samples of pooled urine from rats administered the low molecular weight fraction of PHMB. Samples of resolved metabolites were analyzed using a Finnigan TSQ7000 mass spectrometer. Pools of 0-24 and 0-72 hour post-dose urine were analyzed.

The data were presented in Figure 8, page 42 of the report. These data showed that the urine samples co-chromatographed in the region of the low molecular weight PHMB fraction. The 0-24 hour male and female urine pools as well as the 0-72 hour female urine pool showed evidence of a single metabolite peak. The 0-72 hour male urine pool showed evidence of more than one metabolite. The metabolites were not identified in this study, due to the small mass available for analysis.

Fecal extraction of pooled samples from male rats given a dose of low molecular weight fraction PHMB showed tha less than 14% of the total fecal radioactivity was extractable by methanol or acetonitrile/acetic acid. Since the bulk of fecal radioactivity remained unextracted, no further analysis was performed.

IV. DISCUSSION

The above special metabolism studies were conducted in response to a request by the Agency to investigate the bioavailability and excretion of PHMB in rats (July 22, 1992 memorandum from Timothy F. McMahon to Kathryn Scanlon). The first study examined bioavailability of PHMB following dietary administration at dose levels corresponding to those in the two-year study in rats (ongoing). Rats (8/sex) were fed either 200ppm or 2000ppm PHMB in the diet for 14 days, followed by a single oral dose of 0.08mg or 0.8mg 14-C PHMB as a 9% suspension in the dose vehicle, and excretion measured. As only a minor amount of PHMB-derived radioactivity is excreted in bile, the absorbed dose can be considered as the amount excreted in urine plus residual carcass following removal of the g.i. tract and contents. Based on the data in this study, it can be concluded that at 200ppm dietary administration in

rats, 4.7% of the dose and 3.9% of the dose was available for absorption in male and female rats, respectively. At the 2000ppm dose level, the percentage of the dose available for absorption was 3.0% in male rats and 2.6% in female rats. According to the report, the sex-related difference in absorption was statistically significant at $p < 0.05$ at the high dose of PHMB. In addition, the percentage of the dose absorbed was significantly lower for both sexes at the high dose vs the low dose. It is noted that this study used full molecular weight PHMB, in contrast to the second study, which used various molecular weight fractions of PHMB.

In the second study, three separate experiments were conducted. The first experiment examined biliary excretion of PHMB-derived radioactivity in bile duct cannulated rats after administration of an oral dose of 20 mg/kg unfractionated PHMB. This experiment showed that only between 0.12-0.14% of an administered dose of PHMB is excreted through the bile. Thus, the fecal radioactivity observed (which represents the majority of PHMB-derived radioactivity) is the result of unabsorbed test material. A previously reviewed metabolism study (MRID #'s 00077926 and 00086363) showed feces to be the major route of excretion after a 100 mg/kg dose.

In the second experiment, separate groups of male rats received low, mid, and high molecular weight fractions of PHMB at single oral doses of 20 mg/kg to compare absorption of these various fractions. The mean molecular weight of the low, mid, and high molecular weight fractions of unlabelled PHMB (from fractionation of PHMB by microfiltration using membranes with molecular weight cutoffs of 1k, 3k, or 10k) was: low, 1230; mid, 2070; and high, 3800. The molecular weight of the low, mid, and high fractions for the radiolabelled PHMB were slightly higher: low, 1233; mid, 3048; and high, 4700. Although the average molecular weights for the fractionated radiolabelled PHMB were higher, the report stated that the relative proportions of the monomer, dimar, and trimer and tetramer in both the unlabelled and labelled PHMB were similar. Excretion of PHMB-derived radioactivity in the low, mid, and high molecular weight fraction dose groups showed that the greatest urinary excretion (and hence by inference absorbed dose) was greatest for the low molecular weight fraction dose group, where 5.22% of the administered dose was excreted in urine, vs. 0.15% and 0.22% urinary excretion for the mid- and high-molecular weight fractions.

The third experiment examined the excretion of a low-molecular weight fraction of PHMB, as the second experiment showed absorption to be greatest for this fraction. In male and female rats, administration of a single radiolabelled 20 mg/kg dose resulted in 7.84% of the dose eliminated in urine of male rats, and 2.55% of the dose eliminated in urine of female rats. This result, while consistent with the earlier observation that the bioavailable dose is greater in male vs female rats, is not consistent with the results of the second experiment in study #2, in which a slightly higher percentage of full molecular weight range PHMB was excreted in urine of female vs male rats. As the low molecular weight range material is believed to be the fraction which is absorbed, bile cannulation in rats dosed with full molecular weight range PHMB may result in preferential biliary excretion of low molecular weight range PHMB in male rats. Overall, however, the differences in bioavailable dose between male and female rats do not appear to be marked.

Tissue retention of PHMB derived radioactivity in those rats given a low oral radiolabelled dose was most significant in the liver and kidneys of male and female rats. In males, the concentrations in liver and kidney were 0.568 μg equivalents per gram and 0.499 μg equivalents per gram, respectively. In females, the concentrations in liver and kidney were 0.752 μg equivalents per gram and 0.807 μg equivalents per gram, respectively. Expressed as a percentage of the dose, the liver of male and female rats contained 0.18% and 0.19% of the dose, while the kidneys contained 0.03% and 0.04% of the dose in male and female rats respectively. In all other tissues examined, the concentration of radioactivity was much lower than that observed in liver and kidney (0.007-0.271 μg equivalents per gram).

In summary, the results of the metabolism studies conducted with PHMB show that absorption after dietary administration of PHMB as well as after a single low oral dose of unfractionated or fractionated PHMB is poor. The highest absorption is observed after administration of low molecular weight fraction PHMB, and male rats appear to absorb a slightly higher percentage of the dose than female rats. Excretion in bile is insignificant, and thus the radioactivity appearing in feces is the result of unabsorbed test material. Although more than one metabolite peak was evident in male urine after PHMB administration, these peaks were not definitively identified.

Classification: The studies when taken together are **acceptable**, and satisfy the §85-1 guideline [OPPTS 870.7485] requirement.