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Safety evaluation of a milk basic protein fraction

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Abstract

Milk products are widely consumed by individuals in the US population in the form of fluid milk and milk-derived products and ingredients. Milk is a good source of calcium, which plays a role in maintaining bone health. In addition to calcium, the whey protein fraction of milk contains basic proteins that have been demonstrated to increase bone metabolism and inhibit bone resorption. A specific basic protein fraction in milk (Milk Basic Protein; MBP) was tested in an acute oral toxicity study, teratology study, subchronic oral toxicity study, and reverse mutation assay and no treatment related adverse effects were found. MBP has been evaluated for its use as an ingredient in food and concluded to be safe for its intended use.

Keywords: Milk basic protein; Food; Safety; Ames assay; Teratology; Subchronic toxicity

1. Introduction

Milk basic protein (MBP) is a well-characterized basic protein fraction derived from milk. MBP is approximately 97% total protein. Most of this protein is lactoferrin and lactoperoxidase, which comprise approximately 54% and 41% of the total MBP protein, respectively; other active, basic proteins and other milk proteins are approximately 2.4% of MBP proteins. Studies conducted using the whey protein concentrate (WPC) fraction of milk and the fraction of WPC known as MBP have shown that whey proteins and, more specifically basic protein components of the whey fraction, are important for bone health. This activity has been demonstrated *in vitro* and *in vivo* to strongly stimulate bone formation and inhibit bone resorption (Kato et al., 2000; Takada et al., 1996, 1997a,b,c, 2001; Toba et al., 2000).

Protein components of MBP that have been shown to increase bone metabolism and inhibit bone resorption have been identified. The increase in bone metabolism occurs through an increase in the number of osteoblastic cells and the amount of bone proteins, such as collagen. Two components of MBP that have this growth-promoting activity have been purified and sequenced. One component is identified as a high-mobility group (HMG)-like protein, the other as kininogen fragment 1·2 (Yamamura et al., 1999, 2000). MBP also suppresses bone resorption. The component identified as having this activity is cystatin C (Matsuoka et al., 2002).

The US population has a long history of exposure to MBP constituents through consumption of fluid milk and milk-derived ingredients. The 2005 United States Department of Agriculture (USDA) Dietary Guidelines (USDA, 2005) recommend the consumption of 3 cups of milk or milk products per day by individuals in the US population, in order to promote adequate protein and calcium intake.

Abbreviations: MBP, milk basic protein; WPC, whey protein concentrate; HMG, high-mobility group; USDA, United States Department of Agriculture; mEq, milliequivalents; mg, milligram; kg, kilogram; μg, microgram; μL, microliter; mL, milliliter; NOAEL, No Observed Adverse Effect Level.

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In individuals 31–50 years of age, this recommended intake is an increase of 1.2–1.6 cups of milk (or milk products) per day compared with actual intake by individuals in this age group in the years 2001 and 2002 (USDA, 2005).

Similarly, in Japan, there is a history of exposure to MBP constituents through consumption of fluid milk and milk-derived ingredients. A program established in 2003 by Japan Dairy Association, 3-A-Day Japan, recommends the consumption of 200 ml of milk (one glass of milk), 100 g of yogurt and 20 g of cheese per day by individuals in the Japanese population based on Health Japan 21 (Ministry of Health, Labour and Welfare, 2000) and Dietary Guidelines (Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labour and Welfare, and Ministry of Agriculture, Forestry and Fisheries, 2000). Actual average daily intakes of milk, yogurt and cheese in the year 2002, however, are estimated 95 ml, 20 g and 5 g, respectively using the Statistics on Milk and Dairy Products (Ministry of Agriculture, Forestry and Fisheries, 2002a) and Food Balance Sheet (Ministry of Agriculture, Forestry and Fisheries, 2002b). Based on these assessments, consumption of milk or dairy products in the USA and Japan do not appear to be at optimal levels. Consumption of MBP can supplement the diet of individuals with a specific whey fraction of milk that has been associated with bone health.

As part of the evaluation of the safety of ingestion of MBP, *in vivo* and *in vitro* toxicology studies were completed, including an acute oral study in rats, a teratology study in rats, 13-week oral study in rats, and a reverse mutation assay using *Salmonella typhimurium*.

2. Materials and methods

2.1. Test substance

MBP is a specific basic protein fraction derived from pasteurized skim milk. The total protein content of MBP is greater than 90.0% by weight. Principal protein components are lactoferrin, lactoperoxidase, cystatin C, HMG-like protein and kininogen fragment 1 · 2. MBP was dissolved in water for mutagenicity studies. In the *in vivo* studies, MBP was administered via gavage at a dose volume of 10 mL/kg body weight. Water was used as the control in the mutagenicity and *in vivo* studies.

2.2. Acute oral toxicity in rats

The study consisted of one treatment group and one control group, each with 10 rats per sex per group. Healthy Crj:CD (SD) IGS rats (Charles River Japan, Inc.), approximately 5 weeks of age, were used. At the start of the study, the males weighed between 151 and 165 g and the females weighed between 123 and 138 g. Animals were randomly assigned to study groups based on stratified body weight to ensure that the mean body weight would be similar between the two groups.

MBP was administered at a dose of 2000 mg/kg body weight. The animals were fasted 17–18 h before administration and 4 h after administration. All animals were observed daily from Day 1 to Day 14, on which day each animal was sacrificed in preparation for necropsy.

2.3. Teratogenicity in rats

Healthy male and female Crj:CD (SD) IGS rats were obtained from Charles River Japan, Inc. and mated; males were approximately 12 weeks of age, females were approximately 11 weeks of age. The study utilized one treatment group and one control group; 20 healthy pregnant females were included per group. Copulation was confirmed by the presence of a vaginal plug or sperm in a vaginal smear. The day of copulation was designated as gestation Day 0.

MBP was administered daily by gavage at a dose of 2000 mg/kg body weight/day on Days 7–17 of gestation. All dams were observed for mortality and clinical effects from Day 0 of gestation until necropsy; twice daily during the MBP administration period and once daily before initiation and after completion of MBP administration. Body weights were recorded on Day 0, Day 3, and daily from Day 7 to Day 20 of gestation. Food consumption was measured on Days 0, 3, 7, 9, 11, 13, 15, 17, and 20. Necropsy was performed on Day 20 of gestation. At necropsy, selected organs and tissues were preserved. Ovaries and uteri were removed and the gravid uteri were weighed. After observation of intrauterine, embryo-fetal, and placental conditions, and after the removal of live fetuses, the uteri and placentas were weighted. Implantation index, viability index of fetuses, incidence of dead or resorbed embryos and fetuses, and sex ratio were calculated. The fetal examination consisted of external examination, visceral examination, and skeletal examination. For visceral examination, all fetuses were fixed in Bouin's solution; for skeletal examination, all fetuses were fixed in 99.5% ethanol, stained with alizarin red S and cleared in 70% glycerin. This study was conducted under "Guidelines for Designation of Food Additives and for Revision of Standards for Use of Food Additives", Notification No. 29 of the Environmental Health Bureau, Ministry of Health and Welfare, Japan, March 22, 1996.

2.4. Thirteen-week oral rat toxicity study

A 4-week oral gavage range finding study in rats was conducted to set the MBP dose levels for a subsequent 13-week oral toxicity study in rats. Based upon the results of this 4-week study, MBP doses of 0, 200, and 2000 mg/kg body weight/day were administered for 91 days via gavage to groups of 10 male and 10 female Crj:CD (SD) IGS rats (Charles River Japan, Inc.). Body weight and food consumption were measured and recorded on Days 1, 2, and 7, and weekly thereafter. The general physical condition of each animal was observed during the study once per day. In Week 13, urinalysis (i.e., pH, protein, glucose, ketone body, urobilinogen, bilirubin, occult blood, urinary sediment, volume, specific gravity, sodium, potassium, and chloride) and ophthalmologic examinations were conducted. Before necropsy, a blood sample was collected from the abdominal aorta of each animal under anesthesia for hematological evaluations and clinical chemistry evaluations, specified in Table 1. Necropsies were performed on all animals at the termination of the study and weights were determined for the following organs: heart, liver, spleen, kidneys, adrenals, prostate, testes, seminal vesicles (including the coagulating glands), ovaries, uterus, brain, pituitary, salivary glands (including submandibular and sublingual glands), thymus, lung, and thyroid (including parathyroid).

Histopathological examinations were performed for all animals in the control and high dose groups. Organs and tissues examined are listed in Table 1. Most tissues were fixed and preserved in 10% neutral buffered formalin; eyeballs and harderian gland were fixed and preserved in Davidson's fixative; testes and epididymides were fixed in Bouin's solution and preserved in 70% ethanol.

One-way parametric ANOVA with Dunnett's test was used to compare body weights, body weight gain, food consumption, feed efficiency, quantitative parameters of urinalysis (except specific gravity), hematological values, blood chemistry values, and organ weights. The Kruskal–Wallis test with the Mann–Whitney *U*-test was used to analyze the qualitative parameters of urinalysis and specific gravity.

This study was performed in accordance with "Ordinance on Standard of Conduct of Non-clinical Studies of Drug Safety", Ministry of Health and Welfare Ordinance No. 21, Japan, March 26, 1997 and "Guidelines for Designation of Food Additives and for Revision of Standards for Use of Food Additives", Notification No. 29 of the Environmental Health Bureau, Ministry of Health and Welfare, Japan, March 22, 1996.

Table 1
Parameters evaluated

Hematological and biochemical parameters evaluated
Red blood cells, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, reticulocytes, white blood cells, neutrophils, eosinophils, basophils, lymphocytes, monocytes, prothrombin time, activated partial thromboplastin time, total protein, albumin, albumin to globulin ratio, protein fraction, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, total bilirubin, glucose, total cholesterol, triglyceride, urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorous
Organs and tissues examined
Skin, mammary gland, mandibular lymph nodes, mesenteric lymph node, thoracic aorta, submandibular glands, sublingual glands, parotid glands, sternum, femur, thymus, trachea, lung (including bronchus), heart, thyroids, parathyroids, tongue, larynx, esophagus, stomach (including forestomach and glandular stomach), duodenum, jejunum, ileum (including agmen peyerianum), cecum, colon, rectum, liver, pancreas, spleen, kidneys, adrenals, urinary bladder, seminal vesicles (including the coagulating glands), prostate, testes, epididymides, ovaries, oviducts, uterus, vagina, brain (including cerebrum and cerebellum), pituitary gland, sciatic nerve, skeletal muscle, spinal cord, nasal cavity (turbinate), eyeballs, harderian glands, and Zymbal's glands

2.5. Mutagenicity

MBP was tested for the potential to induce point mutations in a reverse mutation assay using *S. typhimurium* strains TA98 and TA100. All tests were conducted in the presence and absence of metabolic activation (i.e., rat liver homogenate, S9). MBP concentrations of 1.6, 8.0, 40, 200, 1000, and 5000 μg/plate (first test) and 156, 313, 625, 1250, 2500, and 5000 μg/plate (second test) were employed. Negative control samples (i.e., water) were run in triplicate and MBP and appropriate positive control samples were run in duplicate. A positive result was considered to be one that resulted in greater than two-times the number of revertant colonies observed in the negative control plates.

3. Results

3.1. Acute toxicity in rats

No mortality was observed during the 14-day study period. There were no abnormalities in the general appearance in any animal, and no adverse changes in body weight occurred in any animal group during the study. Additionally, no organ pathology was observed at necropsy on Day 14. Accordingly, the median lethal dose (LD_{50}) of MBP for the Crj:CD (SD) IGS rats in this test system was determined to be greater than 2000 mg/kg body weight.

3.2. Teratogenicity in rats

There were no MBP-related adverse clinical effects observed over the course of the teratology study. There were no differences between treatment and control animals in body weight, body weight gain, food consumption, numbers of corpora lutea, numbers of implantation sites, numbers of live and dead fetuses, numbers of resorbed embryos, viability indices of fetuses, sex ratio, placental weight, and body weight of fetuses (Table 2). In live fetuses, there were no significant MBP-related external, visceral, or skeletal anomalies or variations. Based on this study, under these test conditions MBP had no adverse effects on reproduction or development in Crj:CD (SD) IGS rats at 2000 mg/kg body weight/day.

3.3. Thirteen-week oral rat toxicity study

3.3.1. Clinical observations

No MBP-related adverse clinical effects were observed in any animal. One female in the 2000 mg/kg body weight/day dose group exhibited loss of the fourth digit of the right forelimb on Day 49, but this effect was not considered treatment-related.

3.3.2. Body weight

Group mean body weights of male and female animals during the course of the study are shown in Fig. 1. No statistically significant differences in group mean body weight were observed in male animals of either dose group compared to controls. A statistically significant decrease in group mean body weight was observed in female animals in the 200 mg/kg/day dose group on Day 28 compared to controls. This difference was considered anomalous and not treatment-related. A statistically significant decrease in group mean body weight was observed in female animals

Table 2
Reproductive parameters of female rats in MBP teratogenicity study[a]

Parameter (units)	MBP dose groups[b]	
	Control	2000 mg/kg
Number of corpora lutea	16.3 ± 2.0	16.3 ± 2.5
Number of implantation sites	15.8 ± 2.0	15.4 ± 2.2
Implantation index (%)[c]	97.01 ± 4.38	94.84 ± 7.70
Dead or resorbed embryos/fetuses		
Early[d]	1.0 ± 0.7	1.0 ± 1.1
Late[e]	0.0 ± 0.0	0.1 ± 0.3
Total	1.0 ± 0.7	1.1 ± 1.2
Incidence (%)[f]	6.10 ± 4.64	6.55 ± 6.84
Number of live fetuses	14.9 ± 2.0	14.4 ± 1.9
Viability index of fetuses (%)[g]	93.90 ± 4.64	93.45 ± 6.84
Live fetuses		
Sex ratio[h]	0.464 ± 0.153	0.490 ± 0.125
Body weight (g)		
Male	3.720 ± 0.234	3.698 ± 0.209
Female	3.552 ± 0.191	3.568 ± 0.213
Placental weight (g)	0.481 ± 0.038	0.478 ± 0.062

[a] Each value is the group mean ± SD.
[b] $n = 20$ per group.
[c] [Number of implantation sites/number of corpora lutea] × 100.
[d] Includes implantation sites and placental remnants.
[e] Includes macerated fetuses and dead term fetuses.
[f] [Number of dead or resorbed embryos and fetuses/number of implantation sites] × 100.
[g] [Number of live fetuses/number of implantation sites] × 100.
[h] Number of male live fetuses/number of live fetuses.

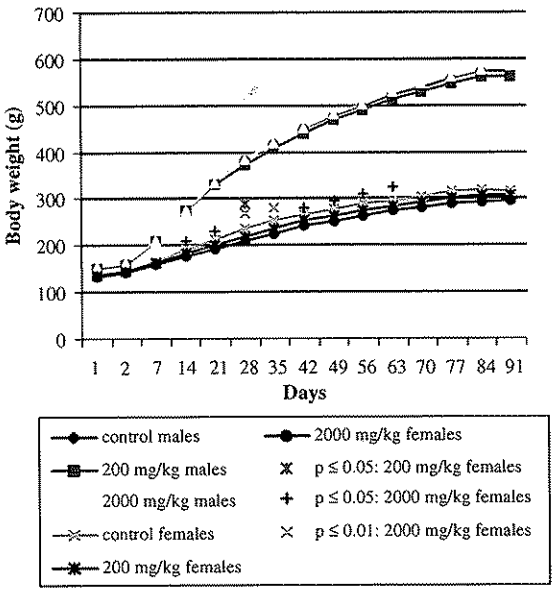

Fig. 1. Body weight during a 13-week toxicity study of MBP in rats. Each value is the group mean, $n = 10$ per group.

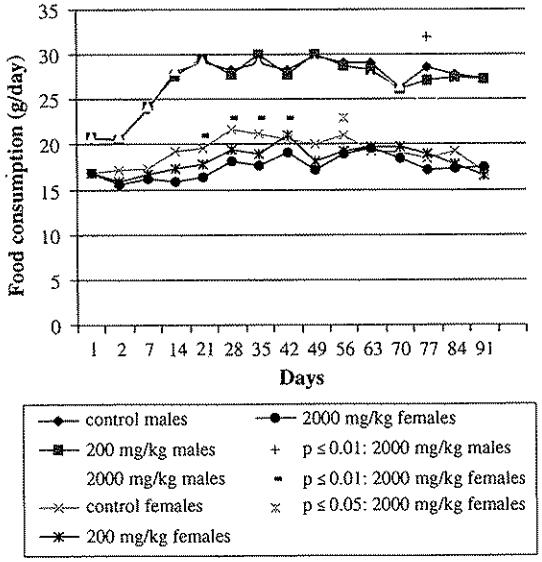

Fig. 2. Food consumption during a 13-week toxicity study of MBP in rats. Each value is the group mean, $n = 10$ per group.

in the 2000 mg/kg/day dose group between Days 14–56 compared with controls. This effect on body weight did not occur in males at this dose and was not seen in the period from Day 63 to the end of the study. A comparison of group mean body weights of female animals from the 2000 mg/kg/day dose group with historical control body weights does not show any treatment related decrease in body weights compared with historical ranges (data not shown).

The following statistically significant differences in group mean body weight gain were observed between treated and control animals: in female animals of the 200 mg/kg/day dose group, an increase on Days 84–91; in female animals of the 2000 mg/kg/day dose group, a decrease on Days 14–21; and in male animals of the 2000 mg/kg/day dose group, an increase between Days 84 and 91. Differences observed in body weight gain between treated and control animals were not consistent over time or across sexes and the changes did not occur in a dose-dependent manner. Over the duration of the treatment period, there was no statistically significant effect on body weight gain of males or females (i.e., between Days 1 and 91).

3.3.3. Food consumption

Group mean food consumption for male and female animals is shown in Fig. 2. No statistically significant differences in group mean food consumption were observed in male or female rats in the 200 mg/kg/day group compared with their respective controls. Male animals in the 2000 mg/kg/day dose group exhibited a statistically significant decrease in group mean food consumption on Day 77, however this finding was considered anomalous and not biologically significant. In female animals in the 2000 mg/kg/day dose group, a statistically significant decrease in group mean food consumption was observed between Days 14 and 35 and on Day 49, which correlated with the period during which these animals had statistically significantly decreased body weights. In female animals, food consumption was not statistically significantly different between controls and the 2000 mg/kg/day dose group from Day 56 to the end of the study. A comparison of the food consumption from females of the 2000 mg/kg/day dose group with historical controls does not show any adverse treatment related effect on food intake compared with historical ranges (data not shown).

3.3.4. Feed efficiency

In the 200 mg/kg/day dose group, statistically significantly increased group mean feed efficiency was observed in female animals between Days 84 and 91, and statistically significantly decreased group mean feed efficiency was observed in male animals between Days 21 and 28. In male animals of the 2000 mg/kg/day dose group, statistically significantly increased group mean feed efficiency was observed between Days 84 and 91; however no differences were observed in female animals of this dose group. Because any differences in feed efficiency were inconsistent over time and between sexes, and they did not occur in a dose-dependent manner, these effects were not considered adverse or treatment-related.

3.3.5. Ophthalmic effects

No adverse or treatment-related ophthalmic effects were observed in any animals in any dose group.

3.3.6. Urinalysis

Urinalysis results (Table 3) showed a dose-dependent increase in group mean sodium and chloride excretion in all animals. The values for sodium excretion, however,

were well within the range of historical controls (i.e., male, from 0.838 ± 0.561 to 1.918 ± 0.300 mEq/21 h; female, from 0.681 ± 0.212 to 1.613 ± 0.417 mEq/21 h) and the values for sodium excretion for the male and female control animals in this study were very low compared with background (i.e., 0.513 ± 0.265 and 0.542 ± 0.213 mEq/21 h for males and females, respectively). Similarly, values for chloride excretion were well within the range of historical controls (i.e., male, from 0.972 ± 0.677 to 2.549 ± 0.391 mEq/21 h; female, from 0.665 ± 0.374 to 2.056 ± 0.445 mEq/21 h) and the values for chloride excretion for the male and female control animals in this study were very low compared with background (i.e., 0.944 ± 0.426 and 0.796 ± 0.288 mEq/21 h for males and females, respectively). Accordingly, the statistically significant differences in sodium and chloride excretion values in the treated animals were considered to be attributed to unusually low control values and not considered to be a biologically adverse finding. No dose-dependent significant effect was seen on potassium excretion in female rats and no significant effects on potassium excretion were seen in male rats.

A statistically significant increase in urinary protein excretion was noted in males and females of the 2000 mg/kg/day dose group. A statistically significant increase in urine specific gravity was observed in males of this dose group. Urinary protein was detected qualitatively using Multistix test paper. It is reasonable to suggest that the qualitative determination of significantly increased protein excretion in the urine with increasing doses of the protein containing test article, MBP, may be due in part to the excretion of protein and not damage to the kidney. This is corroborated by a lack of change in organ weight or histopathology in the kidney.

3.3.7. Hematology

There were no statistically significant differences in hematological values in any animals at any dose group compared with control animals (data not shown). In one

Table 3
Urinary findings in rats administered MBP for 13 weeks[a]

Parameter	Males[b]			Females[b]		
	Control	200 mg/kg	2000 mg/kg	Control	200 mg/kg	2000 mg/kg
pH						
6.5	0	0	0	0	0	1
7.0	0	0	0	1	0	1
7.5	0	0	0	2	2	2
8.0	0	0	1	3	3	2
8.5	10	10	9	4	5	4
Protein						
−	0	0	0	5	2	1
±	7	4	1	5	7	6
+	3	6	6	0	0	3*
++	0	0	3**	0	1	0
Glucose						
−	10	9	10	10	10	10
+	0	1	0	0	0	0
Ketone bodies	10	10	10	10	10	10
Urobilinogen (0.1 EU/dL)	10	10	10	10	10	10
Bilirubin	10	10	10	10	10	10
Occult blood						
−	9	10	9	10	10	9
±	1	0	0	0	0	1
+	0	0	1	0	0	0
Specific gravity						
1.011–1.020	0	1	0	0	0	0
1.021–1.030	4	2	1	1	1	0
1.031–1.040	5	3	3	3	0	4
1.041–1.050	1	4	3	4	8	4
>1.050	0	0	3**	2	1	2
Volume (mL/21 h)	18.65 ± 2.71	18.30 ± 5.97	19.05 ± 6.54	10.10 ± 4.31	10.90 ± 2.02	11.40 ± 3.27
Na (mEq/21 h)	0.513 ± 0.265	0.949 ± 0.464*	1.183 ± 0.333**	0.542 ± 0.213	0.870 ± 0.267*	0.882 ± 0.261**
K (mEq/21 h)	2.525 ± 0.701	2.858 ± 0.722	3.078 ± 0.926	1.741 ± 0.502	2.355 ± 0.392*	1.984 ± 0.740
Cl (mEq/21 h)	0.944 ± 0.426	1.355 ± 0.618	1.864 ± 0.583**	0.796 ± 0.288	1.321 ± 0.348**	1.335 ± 0.443**

[a] Each value is the number of animals (i.e., pH, protein, glucose, ketone body, urobilinogen, bilirubin, occult blood, and specific gravity) or the group mean ± SD (i.e., volume, Na, K, and Cl). Na, sodium; K, potassium; Cl, chloride.

[b] $n = 10$ per group.

* $p \leqq 0.05$.

** $p \leqq 0.01$.

male animal in the 200 mg/kg/day dose group, a high white blood cell count (i.e., 26,900/μL) and an increase in segmented cells (i.e., 27%) was observed, however this effect was considered anomalous and not treatment-related because it was not dose-related or consistent across sexes.

3.3.8. Blood chemistry

Compared with the control group, there were no statistically significant differences in blood chemistry values in male and female animals in the 200 mg/kg/day dose group or female animals in the 2000 mg/kg/day dose group. In male animals in the 2000 mg/kg/day dose group, there was a statistically significant differences in potassium concentration, compared with control animals, however, this effect was not considered biologically significant because it only occurred in males, there was no change in urine potassium excretion values, and there were no abnormalities observed in the kidneys or adrenals in these animals (data not shown).

3.3.9. Necropsy and organ weights

There were no adverse treatment-related effects observed in any animal at necropsy. There were no statistically significant differences in absolute or relative organ weights in any animals at any dose compared with control animals (data not shown).

3.3.10. Histopathology

Histopathologic findings are presented in Table 4. No treatment-related effects were detected from the histological examination of organs. With the exception of one male in the 2000 mg/kg/day group, a low incidence of hyaline casts and cellular infiltration of lymphocytes were observed in both control and treated male and female animals and therefore, these findings were not considered to be treatment-related. One male in the 2000 mg/kg/day dose group exhibited evidence of slight renal alteration reflected in the tubular epithelium with cellular infiltration of lymphocytes. This finding is indicative of infection and not believed to be treatment-related.

In summary, female rats, having received an MBP dose of 2000 mg/kg/day via gavage, exhibited decreases in body weight and food consumption during the study; feed efficiency during this period was not affected. The differences in body weight were not observed on or after Day 63 of administration, but were accompanied by a statistically significant decrease in food consumption between Day 14 and Day 49 (excluding Day 42). The differences in food consumption were not observed on or after Day 56 of administration. In addition, recovery was observed for both food consumption and body weight and observations for these parameters were similar to ranges seen for historical controls. These findings in body weight and food consumption were not observed in male animals of the 2000 mg/kg/day dose group. Therefore, the statistically significant effects noted for body weight and food consumption in female animals of the 2000 mg/kg/day dose group were not considered to be treatment-related.

Statistically significant increases in sodium and chloride excretion in the animals of the 2000 mg/kg/day dose group were considered to be attributed to unusually low control values and not considered to be a biologically adverse finding. A qualitative determination of increased protein excre-

Table 4
Histopathological findings[a]

Female, control										
Animal no.	151	152	153	154	155	156	157	158	159	160
Right kidney: cast, hyaline	–	–	–	–	+	–	–	–	–	–
Left kidney	N	N	N	N	N	N	N	N	N	N
Female, MBP 2000 mg/kg										
Animal no.	351	352	353	354	355	356	357	358	359	360
Right kidney	N	N	N	N	N	N	N	N	N	N
Left kidney	N	N	N	N	N	N	N	N	N	N
Male, control										
Animal no.	101	102	103	104	105	106	107	108	109	110
Right kidney: cast, hyaline	–	–	–	–	+	–	–	–	–	–
Left kidney: cellular infiltration, lymphocyte	–	–	–	–	–	–	–	+	–	–
Male, MBP 2000 mg/kg										
Animal no.	301	302	303	304	305	306	307	308	309	310
Right kidney: cast, hyaline	–	–	–	–	–	–	+	–	–	+
Cellular infiltration, lymphocyte	–	+	–	–	–	–	–	–	–	–
Regeneration, tubular epithelium	–	–	–	–	–	–	–	–	–	+
Dilatation, tubule	–	–	–	–	–	–	–	–	–	+
Left kidney: cast, hyaline	–	–	–	–	–	–	+	–	–	–
Cellular infiltration, lymphocyte	–	–	–	–	–	–	–	–	–	+
Regeneration, tubular epithelium	–	–	–	–	–	–	–	–	–	+

[a] N, no abnormal findings; –, normal; +, slight change.

tion in the urine with increasing dose is attributed to the excretion of protein from the test article, MBP, and is not attributed to damage to the kidney. The conclusion that the protein and electrolyte data are not indicative of direct toxicity is corroborated by a lack of change in organ weight or histopathology of the kidney. Accordingly, based on this study, the no-observed adverse effect level (NOAEL) of MBP in rats was 2000 mg/kg body weight/day.

3.4. Mutagenicity

In both activated and non-activated MBP-treated plates, the average number of revertant colonies was less than two-times the number of revertant colonies observed in the negative control plates, at incubation concentrations up to 5000 μg/plate (data not shown). Results from the positive controls indicated appropriate sensitivity of the test system. There was no MBP concentration-dependent increase in revertants, and MBP-related inhibition of growth was not observed. Based on these results, under these testing conditions MBP was not mutagenic.

4. Discussion

Milk and products derived from milk, such as whey, are widely consumed by Americans of all ages in the form of fluid milk and as milk or milk-derived ingredients. MBP is a specific basic protein fraction derived from pasteurized skim milk. It is intended for use as a dietary ingredient in selected foods and beverages. Given that MBP is produced from milk, the US population is continuously exposed to the constituents of MBP through consumption of milk and milk-containing foods.

As part of a safety determination of this ingredient, MBP has been evaluated in a battery of toxicology tests, including an acute oral toxicity study, teratology study, subchronic oral toxicity study, and reverse mutation assay. Results from these studies were consistently negative. Based on the teratology and subchronic oral toxicity studies, the no-observed adverse effect level (NOAEL) of MBP in rats was 2000 mg/kg body weight/day.

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