



**Memorandum**

Date:     =    FEB 02 2004      
From: Interdisciplinary Scientist/Pharmacist , Division of Dietary Supplement Programs  
      , Office of Nutritional Products, Labeling and Dietary Supplements, HFS-810  
Subject: 75-Day Premarket Notification of New Dietary Ingredients  
To: Dockets Management Branch, HFA-305

Subject of the Notification: **7-hydroxymatiresinol (HMR) potassium acetate complex**

· Firm: Hormos Nutraceutical Oy Ltd

Date Received by FDA: June 30, 2003

90-Day Date: 9/29/03

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification and related correspondence for the aforementioned substance should be placed on public display in docket number 95S-0316 as soon possible since it is past the 90-day date. Thank you for your assistance.

*Maria Chang*

95S-0316

RPT200



SEP 12 2003

Lars Pellas  
Chief Executive Officer  
Hormos Nutraceutical Oy Ltd.  
PharmaCity  
Itainen Pitkakatu 4B  
FIN-20520 Turku  
Finland

Dear Mr. Pellas:

This is to inform you that the notification dated June 27, 2003, you submitted to pursuant to 21 U.S.C. 350b(a)(2)(section 413(a)(2)) of the Federal Food, Drug, and Cosmetic Act (the Act) was filed by the Food and Drug Administration (FDA) on June 30, 2003. Your notification concerns the substance called 7-hydroxymatairesinol (HMR) that you intend to market as a new dietary ingredient. You state that this submission is in response to FDA's letter dated May 23, 2003.

You describe HMR as a substance extracted and purified from Norway spruce tree (*Picea abies*, (L.) H. Karst.b)) wood chips and further processed as hydroxymatairesinol potassium acetate complex (HMR-potassium acetate complex). The dosage form will be oral capsules containing up to 215 milligrams (mg) HMR. The suggested conditions of use are one capsule per day for a recommended maximum daily consumption of 150 mg HMR/day or approximately 3 mg/kilogram (kg) body weight for a 50 kg person and near 2 mg/kg body weight/day for a 70 kg person. You also state that the consumption of the HMR-potassium acetate complex is neither intended specifically for a target population nor required to be excluded from any population sub-group.

Under 21 U.S.C. 350b(a), the manufacturer or distributor of a dietary supplement that contains a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section

Page -2- Lars Pellas, CEO

350b(a)(2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

FDA has carefully considered the information in your submission, and the agency has significant concerns about the evidence on which you rely to support your conclusion that a dietary supplement containing hydroxymatairesinol potassium acetate complex will reasonably be expected to be safe.

According to the notification, a single dose of 150 mg/kg HMR did not result in overt human toxicity. However, the notification states that HMR is recommended for daily consumption. HMR resulted in decreased ovary weights of rats fed 160 mg/kg/d for 90 days. In addition, HMR resulted in increased uterine weights of dogs administered 150 mg/kg/d for 28 days. According to the notification, the increased uterine weights of dogs are not biologically significant since a dose-response effect was not observed. However, the notification makes no statement regarding the biological significance of the decreased ovary weights of rats fed HMR. The notification makes a direct comparison between the doses administered to rats and dogs and the doses recommended for human consumption. The notification does not make any adjustments for the extrapolation of animal data to humans. In addition, the notification does not provide a rationale for recommending a 33% greater dose for 50 kg persons compared to 70 kg persons. Furthermore, HMR induced chromosome aberrations in cultured Chinese hamster ovary (CHO) cells at doses that induce 50% cytotoxicity. These data provide clear evidence that HMR is a clastogen. However, the notification makes no statements regarding the potential implications of chronic clastogen consumption by humans. Based on the data submitted, the notification does not provide sufficient evidence to support the safe use of HMR as a new dietary ingredient.

For the reasons discussed above, the information in your notification does not provide an adequate basis to conclude that HMR-potassium acetate complex, when used under the conditions recommended or suggested in the labeling of your product, will reasonably be expected to be safe. Therefore, your product may be adulterated under 21 U.S.C. 342(f)(1)(B) as a dietary supplement that contains a new dietary ingredient for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such a product into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

Your notification will be kept confidential for 90 days after the filing date of June 27, 2003. After the 90-day date, the notification will be placed on public display at FDA's Docket Management Branch in docket number 95S-0316. Prior to that date, you may wish to identify in writing specifically what information you believe is proprietary, trade secret or otherwise confidential for FDA's consideration.

Page 3 – Lars Pellas, CEO

If you have any questions concerning this matter, please contact Victoria Lutwak at (301) 436-2375.

Sincerely yours,

A handwritten signature in black ink, appearing to be 'SJW', written over a horizontal line.

Susan J. Walker, M.D.  
Acting Division Director  
Division of Dietary Supplement Programs  
Office of Nutritional Products, Labeling  
and Dietary Supplements  
Center for Food Safety  
and Applied Nutrition



June 27, 2003

Susan J. Walker, M.D.  
Acting Director  
Division of Dietary Supplement Programs  
Office of Nutritional Products, Labeling, and Dietary supplements (HFS-820)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5100 Paint Branch Pkwy  
College Park, MD 20740  
USA

Subject: Our New Dietary Ingredient Notification for 7-hydroxymatairesinol potassium acetate complex, filed by the FDA on March 19, 2003.

Dear Dr. Walker,

I refer to your letter dated May 23 regarding our above mentioned New Dietary Ingredient Notification.

In response to your questions we submit the following additional information to augment our notification:

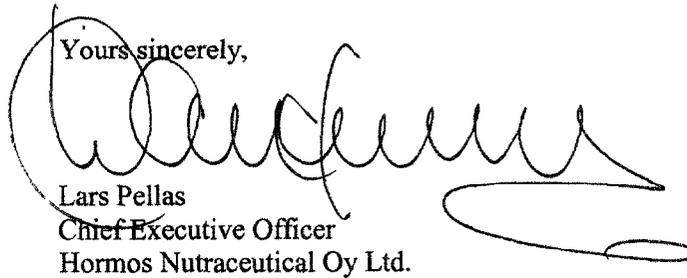
1. The full Latin name of our source Norway spruce, including the Author is:  
(*Picea Abies*, (L.) H. Karst.)
2. Please find enclosed three sets of copies of all referenced studies and publications in accordance with 21 CFR 190.6(b)(4). An updated version of our notification which includes a reference list at the end of the document and relevant citations inserted in the text can be found at the top of each Binder no. 1.

Please be informed that references numbered 1-17, 30-32, 34-35, 38-39 and 41-44 represent proprietary and confidential studies yet to be published and we therefore respectfully request that they will not be made publicly available at FDA's Docket Management Branch or otherwise.

3. Please find enclosed our proposed new wording for section 3 under 21 CFR 190.6(b)(3)(ii).

We understand that our notification was filed by the FDA on March 19, 2003. We further understand that the 75 day period during which we must not introduce or deliver for introduction into interstate commerce for use as a dietary ingredient in any dietary supplement was interrupted on May 23, and will resume again upon receipt of the requested additional information by the FDA. We respectfully request your confirmation of our understanding.

Yours sincerely,

A handwritten signature in black ink, appearing to read 'Lars Pellas', with a long horizontal flourish extending to the right.

Lars Pellas  
Chief Executive Officer  
Hormos Nutraceutical Oy Ltd.

In accordance with the Dietary Supplement Health and Education Act of 1994 (DSHEA), 21 U.S.C. § 350b (a) (2), and with final regulations published in Federal Register 49886-49892), 21 C.F.R. § 190.6 "Requirement for Premarket Notification", the following information is submitted by Hormos Nutraceutical Oy Ltd. in support of a New Dietary Ingredient Notification for 7-hydroxymatairesinol. Hormos Nutraceutical Oy Ltd. intends to market 7-hydroxymatairesinol as a dietary supplement in the United States. As per the statutes of the DSHEA, 21 U.S.C. § 350b (a) (2), Hormos Nutraceutical Oy Ltd. will not introduce, market, distribute or sell 7-hydroxymatairesinol until at least 75 days following official acknowledgement of the receipt of this notification by the U.S. Food and Drug Agency (FDA).

### SECTION 1

**The name and complete address of the manufacturer of the dietary supplement that contains the dietary ingredient, or the dietary ingredient.**

The manufacturer of the dietary ingredient will be:

Hormos Nutraceutical Oy Ltd.  
Pharmacy  
Itäinen Pitkäkatu 4C  
FIN-20520 Turku  
Finland

Attention: Mr. Lars Pellas  
Vice President,  
Corporate Development  
Hormos Medical Corporation  
Pharmacy  
Itäinen Pitkäkatu 4C  
FIN-20520 Turku  
Finland

### SECTION 3

**Description of the dietary supplement or dietary supplements that contain the dietary ingredient including (i) the level of dietary ingredient in the dietary supplement, and (ii) the conditions of use recommended or suggested in the labeling of the dietary supplement, or if no conditions of use are recommended or suggested in the labeling of the dietary supplement, the ordinary conditions of use of the supplement.**

7-Hydroxymatairesinol potassium acetate complex (HMR potassium acetate complex) will be marketed for use in products meeting the definition of "dietary supplement" in section 201 (ff) of the Federal Food, Drug, and Cosmetic Act. The HMR-potassium acetate complex will be clearly labeled and promoted as a dietary supplement. HMR-potassium acetate complex will be sold in the form of oral capsules, each capsule will contain up to 215mg of active ingredient. Consumption of 1 capsule per day will be suggested or recommended, resulting in a recommended maximum daily consumption of 150 mg HMR/day, or approximately 3 mg/kg body weight for a 50 kg person and near 2 mg/kg body weight/day for a 70 kg person. Consumption of the HMR-potassium acetate complex is neither intended specifically for a target population nor required to be excluded from any population sub-group.

## SECTION 4

**The history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe, including any citation to published articles or other evidence that is the basis on which the distributor or manufacturer has concluded that the dietary supplement will reasonably be expected to be safe.**

The overall safety of HMR potassium acetate complex is supported by the results of pharmacology, toxicology and related testing in animals, clinical studies in humans, and by data on other related plant lignans that are consumed directly in conventional food or which are marketed as dietary supplements.

### Animal Studies

The results of unpublished standard general pharmacology studies demonstrate that HMR does not have pharmacological activity<sup>1-10</sup>. Specifically, HMR did not affect mean arterial pressure and heart rate in rats administered doses of up to 30 mg/kg body weight by i.v. injection<sup>1</sup>. There were no effects of HMR on pentobarbital-induced sleeping times in rats dosed by oral gavage at up to 100 mg/kg body weight<sup>2</sup>. Similarly, at this dose level HMR did not alter motor co-ordination<sup>3</sup>, spontaneous motor activity<sup>4</sup>, and gastrointestinal transit time in mice<sup>5</sup>, or rectal temperatures<sup>6</sup>, pain thresholds<sup>7</sup>, and kidney function in rats<sup>8</sup>. There were no overt effects of HMR, administered orally at doses of up to 1,000 mg/kg body weight, in male NMRI mice in the Modified Irwin Screen test<sup>9</sup>. In telemetered dogs treated by oral gavage with single HMR doses of 2, 20, and 200 mg/kg body weight, there were no obvious effects on respiratory rate, tidal volume, or minute volume<sup>10</sup>. Electrocardiograms showed no effects of HMR on P-wave amplitude, P-wave duration, P-Q interval, QRS interval, or Q-T interval<sup>10</sup>.

The toxicity of HMR has been extensively studied in animals. One acute toxicity study<sup>11</sup>, 3 subchronic toxicity studies (14-, 28-, and 90-day studies)<sup>12, 13, 14</sup>, and a teratology study<sup>15</sup> have been performed in rats. A 15-day<sup>16</sup> and a 28-day study<sup>17</sup> have also been conducted in beagle dogs.

In an unpublished acute toxicity study<sup>11</sup> single gavage doses of 1,000 and 2,000 mg HMR/kg body weight were administered to groups of male and female rats. Signs of toxicity were then monitored for 14 days. The test was performed in accordance with Organization for Economic Co-operation and Development (OECD) guidelines for acute oral toxicity testing. There were no deaths or other signs of toxicity reported. Similarly, there was no effect of treatment on macroscopic or microscopic observations. The

authors concluded that the minimal lethal dose was above 2,000 mg HMR/kg body weight.

In the 28-day subchronic rat study<sup>13</sup> HMR was administered by oral gavage to groups of 10 male and 10 female Sprague-Dawley rats at doses of 0, 239, 477, and 955 mg/kg body weight/day, and 0, 242, 483, and 967 mg/kg body weight/day for the first 18 and the second 15 days, respectively. An additional 3 animals/sex/group were included for toxicokinetic analyses. There were no treatment-related effects on body weight, body weight gains, food consumption, organ weights, or on hematological, clinical chemistry, and urinalysis parameters. Four high-dose females, 1 high-dose male, and 1 mid-dose female died likely due to a relatively large volume of a bolus dose combined with a thick consistency of the test article formulation. There was no effect of treatment on the incidence of findings at pathological examination, except for the finding of a large thyroid in 1 high-dose male. Histopathological examination indicated the presence of slight follicular cell hypertrophy in high-dose males. The relationship of this finding to treatment was unknown; however, since no indications of thyroid follicular cell hypertrophy were seen in a 90-day study<sup>14</sup> in which rats were treated with HMR at dose levels of up to 2,600 mg/kg body weight/day, the finding in the 28-day study was considered incidental. In any case, oral gavage administration of HMR at doses of up to 967 mg/kg body weight/day for 28 days was well tolerated without overt toxicity.

In a preliminary "Maximum Tolerated Dose" study<sup>16</sup> HMR was administered in capsule form to 1 male and 1 female beagle dog at escalating doses of 0, 200 (days 1-4), 500 (days 5-7), and 700 (days 8-11) mg/kg body weight/day for 11 days. At the end of dosing, the control animals were dosed at 700 mg/kg body weight/day for 15 days. A second formulation of HMR was tested in capsule form at a dose of 636 mg/kg body weight/day for 5 days in one female and one male dog used in the MTD phase of the study. The MTD phase of the study demonstrated that 700 mg/kg body weight/day was well tolerated. Dosing at 700 mg/kg body weight/day for 15 days produced no treatment-related effects on clinical signs, mortality, body weight, body weight gain, food consumption, hematology or clinical chemistry parameters, or on the result of organ weight assessment and macroscopic examinations.

HMR was subsequently tested in groups of 3 male and 3 female beagle dogs by daily administration in capsule form at doses of 0, 146-149, 341-347, and 682-685 mg/kg body weight/day for 28 days<sup>17</sup>. There were no treatment-related effects on mortality, clinical signs, hematological or clinical chemistry parameters, urine analysis, organ weight data, or on the results of electrocardiographic examinations. The group mean body weight of the high-dose males was found to be consistently lower than controls at pre-dose and throughout the study period. Similarly, in high-dose animals, food consumption appeared slightly decreased compared to controls. Food consumption did increase in these groups as the study progressed. The mean absolute and relative uterine weights appeared increased in dosed females; however, given the lack of dose-response or of histopathological correlates, and noting that all of the values were within the historical control ranges, this finding was not considered to be related to HMR treatment. Histopathological examination revealed a single healing erosion in the ileum of 1 high-

dose female dog (correlating to a red, depressed area noted at gross necropsy) and of follicular cell hypertrophy in 1 male and 1 female of the high-dose groups. Due to the small number of animals used in this study, the relationship of these macroscopic and histopathological findings cannot be ascribed to treatment, but neither can a treatment-related effect be discounted.

In an unpublished subchronic toxicity study<sup>14</sup> groups of 20 male and 20 female Wistar rats were fed HMR in the diet at concentrations of 0, 0.25%, 1.0%, and 4.0% for 13-weeks. These dietary concentrations resulted in HMR intakes of approximately 0, 160, 640, and 2,600 mg/kg body weight/day. There were no effects of HMR treatment on mortality, neurobehavioral observations, motor assessment results, ophthalmoscopic examinations, urinalysis parameters, sperm analysis, or on the results of the macroscopic examinations. Histopathological examination revealed a decreased incidence of hyaline droplet nephropathy, a beneficial effect, in high-dose males.

Male rats dosed at 4.0% in the diet showed decreased body weights throughout the study period. In mid-dose males and in mid- and high-dose females, this effect occurred only in the first few weeks of the study. In both sexes treated at the high-dose, there was an increase in the number of animals with sparsely haired skin. Hematological analyses at the end of the study revealed increased thrombocytes in high-dose females and increased WBC count and absolute neutrophil count in high-dose males. Several clinical chemistry changes were noted, including: decreased cholesterol and fasting glucose in high-dose males, increased albumin and A/G ratio in high-dose males, increased GGT in high-dose females, decreased triglycerides in treated males, decreased phospholipids in mid- and high-dose males, and increased total bilirubin in mid- and high-dose males. The clinical chemistry changes, in particular the findings of reduced cholesterol, triglycerides, and phospholipids, have been noted in response to dietary administration of high fiber concentrations that are metabolized by gut microflora (as with HMR). This metabolism results in production of short-chain fatty acids that stimulate hepatic and peripheral metabolism of carbohydrates and fats<sup>18, 19, 20</sup>.

Several organ weight changes were reported in the treated groups. In treated males and in high-dose females, the relative weight of the filled cecum was increased. Similarly, both the absolute and relative weights of the empty cecum were increased in males at the top 2 dose levels and in high-dose females. This effect is a well-known physiological response to high-dietary concentrations of certain substances that alter the osmotic loading of the lower gut, and as such is not considered adverse<sup>20, 21, 22, 23, 24</sup>. In high-dose males, the relative kidney and testes weights were increased, and the relative weight of the adrenal and the absolute brain weight were decreased, in comparison to controls. Modest decreases (about 10%) in ovarian weights were reported in treated females, with the relative ovarian weight decreased in mid- and high-dose groups. There were no histopathological correlates for any of the reported organ weight changes.

Vaginal smears taken during the last 3 weeks prior to sacrifice demonstrated that the number of high-dose females with a maximum estrous cycle length of 4 days was decreased, while those with a maximum cycle length of 5 days was increased, in

comparison to controls. This finding was also reflected in an increased mean estrous cycle length in this dose group.

The effects of HMR on ovarian weights and on estrous cycle length could be indicative of a very weak antiestrogenic effect consistent with stronger such effects known for many other phytoestrogens, including soy isoflavones and secoisolariciresinol diglycoside (SDG) derived from flaxseed<sup>25, 26, 27</sup>. These effects may be related to the metabolism of HMR to enterolactone<sup>25</sup>. Minor changes in ovarian weights and in estrous cycle length are not adverse and are not indicative of strong estrogenic potency. This is evidenced by the lack of estrogen receptor binding of HMR in an *in vitro* study. The study authors reported a "No Observable Effect Level" (NOEL) of 0.25% in the diet, or, for Wistar rats, a dose of approximately 160 mg/kg body weight/day.

The teratogenic potential of HMR has been evaluated in an unpublished prenatal developmental toxicity study<sup>15</sup>. HMR was administered in the diet to groups of 24 mated female Wistar out bred rats at concentrations of 0, 0.25%, 1.0%, and 4.0% from gestational day 0 (fertilization) through until gestational day 21. These dietary concentrations equated to HMR intakes of approximately 0, 140-180, 460-740, and 1,190-2,930 mg/kg body weight/day in the control through high-dose groups, respectively. At Caesarean section dams and fetuses were macroscopically examined and the fetuses, placental material, reproductive organs, and the full and empty cecum weighed. Fetuses were further evaluated for both visceral and skeletal abnormalities.

Thirteen of the high-dose animals (out of 24) were sacrificed due to the fact that they either did not eat or ate less than 4 g of food per day. There were no differences in the clinical signs between the treated and control groups. Both body weight and food consumption were significantly decreased in high-dose animals from gestational day 3 through until Caesarean section. This was most probably related to poor palatability of the diet. Necropsy of the dams did not reveal any treatment-related pathological changes. The absolute and relative weights of the full cecum were increased in the high-dose group while the absolute and relative weights of the empty cecum were increased in both the mid- and high-dose groups. There were no pathological changes associated with the increased cecal weights. The study authors concluded that the maternal NOEL was at 1.0% in the diet or at least 460 mg/kg body weight/day based on a decreased body weight at the top dose.

There were no effects of treatment on reproductive indices including female fecundity, number of corpora leutea, implantation sites, number of live fetuses, number of early and late resorptions, sex-ratio, and amount of pre- and post-implantation loss. External evaluation of the fetuses and placental material revealed no effects of treatment. The weights of the fetal females and of all fetuses combined were decreased from dams of the high-dose group. There were no treatment-related effects on the incidence of either visceral or skeletal abnormalities. The incidence of kinked ureter was increased in fetuses from the mid- and high-dose dams; however, this particular finding is generally considered a variation<sup>28</sup> essentially harmless<sup>29</sup> result, the study authors concluded that

the NOEL for developmental toxicity was at least 4.0% in the diet (at least 1,190 mg/kg body weight/day), the highest dose tested in the study.

The genotoxicity of HMR was evaluated in 3 unpublished studies sponsored by Hormos Nutraceutical Oy Ltd., including an Ames bacterial mutagenicity assay<sup>30</sup>, an *in vitro* chromosome aberration assay<sup>31</sup> and an *in vivo* rat bone marrow micronucleus study<sup>32</sup>. All 3 of these studies were conducted according to OECD protocols and were consistent with U.S. FDA Redbook guidelines.

In the Ames assay<sup>30</sup>, the test substance in dimethylsulfoxide (DMSO) was not toxic to *Salmonella typhimurium* strain TA100 at concentration levels of 1.6, 8, 40, 200, 1,000, or 5,000 µg/plate. Similarly, HMR was not toxic to *Salmonella typhimurium* strains TA98, TA102, TA1535, or TA1537 at concentrations of 5,000 µg/plate. Following incubation with HMR, both in the absence and in the presence of an exogenous source of metabolic activation (Aroclor 1254-induced liver post-mitochondrial fraction (S9) from Sprague-Dawley rats), there were no biologically or statistically significant increases in the number of revertant colonies in comparison to solvent treated controls.

HMR was tested in an *in vitro* cytogenetic assay using Chinese hamster ovary cells<sup>31</sup>. In one set of experiments, HMR was incubated, either in the presence or absence of an exogenous source of metabolic activation (rat S9), for 3 hours followed by a 17-hour recovery period. Concentrations tested ranged from 0 to 1,083 µg/ml (without S9) and from 0 to 1,227 µg/ml (with S9). At the highest concentrations tested in each case there was a significant increase in the number of cells with chromosomal aberrations compared to solvent treated controls. However, at these concentrations, cell numbers were decreased by 51 and 59% in the presence and absence of S9, respectively. This indicates that concentrations associated with chromosomal aberrations were also associated with significant cytotoxicity. In a second series of experiments, treatment was repeated in the absence and in the presence of S9 for 3 hours plus a 17-hour recovery period. As in the first experiment, the highest concentrations tested (862.4 µg/ml and 1,253 µg/ml in the absence and presence of S9, respectively) were associated with an increase in the frequencies of structural aberrations. However, as before, at these concentrations, a 51 to 59% reduction in cell survival was reported. As a result, the study authors concluded that HMR could induce chromosome aberrations, but only at concentrations inducing at least 50% cytotoxicity.

In the *in vivo* rat bone marrow micronucleus assay, groups of 6 male Charles River CD rats were treated with HMR by corn oil gavage at doses of 0, 500, 1,000 and 2,000 mg/kg body weight once daily for 2 consecutive days<sup>32</sup>. Doses were established on the basis of an initial toxicity study in 3 male and 3 female rats. The rats were killed 24 hours after the second administration. Treatment with HMR did not increase the group mean ratios of polychromatic: normochromatic erythrocytes above the values reported for the negative controls or in comparison to historical control ranges. Similarly, the frequencies of micronucleated polychromatic erythrocytes were unaffected by treatment. The positive control, cyclophosphamide, administered by gavage in saline at a dose of 20 mg/kg (single dose) produced the expected increase in micronucleated polychromatic

erythrocytes. Based on these results, the study authors concluded that HMR did not induce genotoxic effects in this assay system. These results also support the conclusion that chromosomal aberrations reported in the *in vitro* CHO study are solely due to cytotoxic effects.

Taken together, the results of the 3 genetic toxicity studies indicate that HMR is without intrinsic genotoxic potential.

The metabolism of HMR was evaluated in a published study<sup>33</sup> in rats. Rats were administered HMR by oral gavage at doses of 3, 15, 25, and 50 mg/kg body weight on 2 consecutive days during which time they were housed in metabolic cages and urine collected for HPLC analysis of metabolites. Enterolactone was the major metabolite identified reaching levels in the urine that were more than 8-fold those found in controls. Other metabolites detected included hydroxyenterolactone,  $\alpha$ -conidendrin, conidendric acid, enterodiol, allo-HMR, and unchanged HMR. No analysis of potential fecal metabolites was conducted. All of these minor metabolites, including unchanged HMR, were at concentrations many-fold (usually greater than 10-fold) lower than the primary metabolite enterolactone.

In another unpublished study<sup>34</sup> designed to evaluate the absorption and tissue distribution of HMR, tritiated-HMR was administered to groups of 2 male and 2 female Sprague – Dawley rats by both oral gavage and intravenous injection. Following oral administration at 250 mg/kg body weight, measures of total radioactivity indicated that HMR was well absorbed, with peak plasma concentrations occurring approximately 1 hour post-dosing. Non-volatile radioactivity was reported to be well distributed to body tissues with the highest concentrations found in the gastrointestinal and urinary systems. Rapid clearance of total radioactivity (concentrations in tissues and plasma less than 10% of peak values at 24 hours post-dose) was reported, indicative of rapid excretion in the urine. By comparing AUC values for plasma concentrations from the oral study with AUC values obtained following dosing *via* intravenous injection (25 mg/kg body weight), the authors concluded that about 56% of the initial radiolabeled dose was absorbed following oral administration. In addition, it was apparent that the sites of the radiolabel in parent HMR were stable as there was very minimal exchange of radiolabel with body water.

The results of the *in vivo* metabolic studies are complimented by an *in vitro* study<sup>35</sup> in which HMR, as the individual diastereoisomers HMR and allo-HMR, was incubated in the presence of human or rat liver homogenate preparations. Both HMR and allo-HMR were rapidly metabolized by the human liver homogenate (*i.e.*, about 80%-90% cleared within 60 minutes), with glucuronidation comprising the most significant biotransformation process, accounting for more than 95% of the metabolites generated. Compared to the human liver homogenate, rat liver preparations were much less efficient in clearing either HMR isomer. Only about 30%-60% was cleared within 60 minutes by the rat liver preparation. The metabolite profile and the active biotransformation processes, however, were similar between the 2 species. In contrast to the results of the *in vivo* study in rats, enterodiol and enterolactone metabolites were not found to occur

following incubation with either the human or rat liver homogenate *in vitro*. This result is expected given the recognized conversion of plant lignans into their mammalian counterparts (enterolactone and enterodiol) by way of facultative intestinal microbes<sup>36,37</sup>

In addition to the preceding standard pre-clinical studies, Hormos Nutraceutical Oy Ltd. sponsored several specialized studies to investigate the antioxidant<sup>38,39</sup>, chemo-preventive<sup>33,40,41</sup> and potential estrogenic activity of HMR<sup>42</sup>.

The results of the unpublished investigations of the antioxidant properties of HMR, both *in vitro* and *in vivo*, showed that this substance was an effective antioxidant. *In vitro*<sup>38</sup>, HMR: a) inhibited lipid peroxidation induced by tert-butylhydroperoxide (IC<sub>50</sub> of 0.06 µmol/L), b) inhibited oxidation of human low-density lipoprotein (LDL) by copper (IC<sub>50</sub> of 6.7 nmol/mg LDL), c) inhibited LDL oxidation due to incorporation into LDL particle (IC<sub>50</sub> of 130 nmol/mg LDL), d) scavenged superoxide anions produced by xanthine-xanthine oxidase (EC<sub>50</sub> of 5.6 µmol/L), and e) scavenged peroxy radicals generated by thermal decomposition of 2,2'-azobis(2-amidinopropane) hydrochloride (1 mole of peroxy radical scavenged per 4 moles of HMR). Minor metabolites of HMR, including α-conidendrin and conidendric acid also showed significant antioxidant activity in the above *in vitro* assays.

In one of the *in vivo* antioxidant studies<sup>39</sup>, weanling male Sprague-Dawley rats were fed a diet containing HMR to provide a dose of approximately 40 mg/kg body weight/day for 5 months. Controls received diets not supplemented with HMR. Following the 5 month feeding period the animals were killed and liver, lung, and kidney tissues examined for signs of oxidative stress, specifically diene conjugation as an indication of lipid peroxidation. HMR had no effect on any measures of oxidative stress. In a second experiment, oxidative stress was induced experimentally in male mice through combined vitamin-E deficient diets and administration of carbon tetrachloride. Test diets contained either HMR (300 ppm or about 50 mg/kg body weight/day) or HMR in combination with α-tocopherol (restored to normal dietary levels). Diets were administered for up to 4 weeks. Oxidative stress was measured through analysis of the presence of thiobarbiturate reactive substances (TBARS) in the liver. HMR alone and in combination was shown to substantially reduce the generation of TBARS (signs of oxidative stress). Also, HMR appeared to potentiate the antioxidant effects of vitamin E.

In a published study of the chemo-preventative effects of HMR<sup>40</sup>, the inhibitory effects of HMR or rye bran on intestinal tumor development was tested in adenomatous polyposis colimultiple intestinal neoplasia (Apc)<sup>Min</sup> mice. HMR, along with inulin (2.5%), was administered to the male mice in a high fat diet, at a concentration of 200 ppm for a period of 5 to 6 weeks. Compared to the control diet (2.5% inulin, high fat diet), and compared to the control diet/rye bran combination, in HMR treated mice the mean number of adenomas in the small intestine was significantly lower (26.6 *versus* 39.6 and 36.0 in mice administered the control diet and the rye bran/control diet, respectively). HMR also appeared to normalize the levels of β-catenin concentrations within the adenomatous tissue. This was considered by the authors to indicate that the chemo-preventative effect of HMR was mediated through the Apc- β-catenin pathway.

The authors did note that although there were no differences in body weight gain amongst the treatment and control groups, mice administered diet supplemented with HMR tended to have an increased amount of hair shedding.

Further chemo-preventative activity of HMR was demonstrated in an unpublished study in which, 3-days following injection of LNCaP prostate cancer cells into both flanks of male nude mice, treatment with HMR in the diet at concentrations of 0.15% and 0.3% for 9 weeks reportedly decreased mean tumor weight and serum concentrations of PSA<sup>41</sup>. Similarly, in a rat mammary tumor model in which mammary tumors were induced by 7,12-dimethylbenz[a]anthracene, treatment with HMR in the diet was reported to inhibit tumor growth at 15 mg/kg body weight/day over a period of up to 17 weeks<sup>33</sup>.

The potential estrogenic effects of HMR were evaluated in an unpublished study<sup>42</sup> on the ability of HMR to competitively inhibit the *in vitro* binding of a fluorescein labeled estrogen receptor ligand (ES2) to estrogen receptors  $\alpha$  and  $\beta$ . Competitive binding was compared to the standard of 17 $\beta$ -estradiol. HMR was found not to competitively bind with ES2 for either estrogen receptors  $\alpha$  or  $\beta$ . The maximum concentration of HMR tested in this assay was 10,000 nM.

#### Human Data

In addition to the aforementioned preclinical safety data, 2 clinical trials have<sup>43</sup> or are<sup>44</sup> being conducted to assess the tolerability, safety, and toxicokinetics of HMR in human subjects.

The results of one clinical trial have been presented in an unpublished report<sup>43</sup>. In this study, single doses (3, 10, 30, 100, 300, 600, and 1,200 mg) of HMR, or a placebo, were given to healthy male volunteers in capsule form. At the 4 lower doses levels (up to and including 100 mg), 3 subjects received HMR and 1 subject received placebo. At the 300 mg dose level, 8 subjects received HMR and 2 subjects received placebo. At the 2 highest dose levels, 5 subjects received HMR and 1 subject received the placebo. Plasma concentrations of HMR, enterolactone, and other minor metabolites of HMR were measured 7, 3, and 1 day prior to HMR/placebo administration to establish baseline values. Following consumption of the capsule, blood was collected for analysis at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 30, 48, and 72 hours post-dosing. Similarly, urine was collected in fractions for the 3 day period following test article administration and analyzed for the presence of HMR and its conjugated and unconjugated metabolites. LDL and DNA oxidation were measured prior to treatment and 2, 6, and 48 hours post-dosing. Safety was monitored through physical examination, clinical chemistry and hematological measurements, ECG recordings, and adverse event reporting (if any).

The pharmacokinetic analysis revealed that peak concentrations of HMR in plasma were reached 0.5 to 2.0 hours post-dosing, regardless of the dose administered. This result is consistent with the pharmacokinetic data obtained in rats.  $C_{max}$  values ranged from 0.29 ng/ml at the 3 mg dose level to 326.86 ng/ml at the 1,200 mg dose level. While the

plasma levels of HMR increased with dose, the increase was often not linear and showed considerable inter-individual variation. Also, concentrations of HMR were detected in the plasma of the placebo group, and, in some cases, these concentrations exceeded those associated with the low dose of 3 mg. Elimination of HMR from plasma was rapid with a  $t_{1/2}$  values ranging from 2.6 to 5.1 hours.

With respect to safety, the study authors concluded that HMR was well tolerated without any report of serious adverse events. There was no effect of HMR on clinical chemistry and hematological evaluations, vital signs, ECG recordings, or on the results of the physical examinations. There were no clear beneficial effects of HMR treatment on LDL oxidation products or the oxidation of DNA as measured by the levels of 8-OH-guanidine.

A second tolerability, safety and kinetic study has been completed<sup>44</sup>, but not report finalized, involving multiple dosing of male volunteers with mild hypercholesterolemia. Also, this study was designed to investigate potential beneficial effects of HMR consumption on lipoprotein oxidation products in this study group. In this study HMR was given to test subjects at daily doses of 315 and 1350 mg (capsule administration, daily doses divided in three equal portions) for 4 weeks. Analysis of plasma HMR and metabolite levels confirmed the efficient adsorption of HMR from the gastro-intestinal tract, as well as an active conversion of HMR into enterolactone. In the absence of any treatment related adverse reactions this study further confirmed the tolerability of HMR potassium acetate preparation after multiple dosing at considerably high doses.

In addition to the clinical studies on HMR, its safety is further corroborated by the history of safe consumption of plant lignans in the diet. Lignans possessing the 2,3-dibenzylbutane carbon skeleton (as with HMR) are ubiquitous in the fiber portion of higher plants. Plant lignans occur in most of the plant parts including roots, leaves, stem, seed, and fruits. As with HMR, intestinal metabolites of these higher plant lignans include enterolactone and enterodiols, 2 lignans that are present naturally in the blood of mammalian species, including humans. A number of plant-derived lignans and similar products (*e.g.*, soy isoflavones) are currently marketed as Dietary Supplements in the United States and have not been associated with the occurrence of adverse effects. Beyond the history of safe consumption of similar plant lignans in the normal diet, there is considerable evidence to suggest that consumption of plant lignans may have antioxidant and chemo-preventative effects. In particular, lignan rich diets are known to elevate enterolactone concentrations in the serum. Reduced enterolactone levels have been associated with increased risk for the development of breast cancer and the occurrence of acute cardiac events.

Based on the preceding information, the use of the new dietary ingredient HMR according to suggested labeling (*i.e.*, maximum daily intakes equivalent to 2 and 3 mg/kg body weight in a 70 and a 50 kg individual, respectively) is concluded to be safe.

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