Final Amended Report

Safety assessment of Oryza Sativa (Rice) Bran Oil, Oryza Sativa (Rice) Germ Oil, Rice Bran Acid, Oryza Sativa (Rice) Wax, Hydrogenated Rice Bran Wax, Oryza Sativa (Rice) Extract, Oryza Sativa (Rice) Bran Extract, Oryza Sativa (Rice) Germ Powder, Oryza Sativa (Rice) Starch, Oryza Sativa (Rice) Bran, Hydrolyzed Rice Bran Protein, and Hydrolyzed Rice Protein

Abstract

Oryza Sativa (Rice) Bran Oil, Oryza Sativa (Rice) Germ Oil, Rice Bran Acid, Oryza Sativa (Rice) Wax, Hydrogenated Rice Bran Wax, Oryza Sativa (Rice) Extract, Oryza Sativa (Rice) Bran Extract, Oryza Sativa (Rice) Germ Powder, Oryza Sativa (Rice) Starch, Oryza Sativa (Rice) Bran, Hydrolyzed Rice Bran Protein, and Hydrolyzed Rice Protein are derived from rice Oryza sativa. ***Oils, Fatty Acids, and Waxes:*** *Rice Bran Oil functions in cosmetics as a conditioning agent and solvent and was used in 34 formulations in 2001. Rice Germ Oil functions as a skin-conditioning agent and was reported in body and hand creams, lotions, powders, and sprays. Rice Bran Acid functions as a surfactant -cleansing agent and was not reported in use. The two Bran Waxes function as binders, conditioning agents, and viscosity increasing agents and were used in 17 formulations. Rice Bran Oil had an oral LD_{50} of > 5 g/kg in white rats and Rice Wax had an oral LD_{50} of > 24 g/kg in male mice. A three-generation oral dosing study reported no toxic or teratologic effects in albino rats fed 10% Rice Bran Oil compared to a control group fed Peanut Oil. In primary dermal irritation studies, undiluted Rice Bran Oil had a PII of 0.00 and 0.88, Rice Wax had a PII of 0.21, and Hydrogenated Rice Bran Wax had a PII of 0.0 (scores > 5.0 were considered irritants). Rice Germ Oil did not produce dermal irritation and Rice Bran Oil was not a sensitizer. Rice Bran Oil, Rice Germ Oil, Rice Wax, and Hydrogenated Rice Bran Wax were negative in ocular toxicity assays. A mixture of Rice Bran Oil and Rice Germ Oil was not phototoxic in a dermal exposure assay. Rice Bran Oil was negative in an Ames assay, and a component, γ-oryzanol, was negative in bacterial and mammalian mutagenicity assays. Animal and clinical studies have reported that consumption of Rice Bran Oil or Rice Bran had protective effects on blood parameters. Formulations containing 1.04% or 8.0% Rice Bran were at most mildly irritating in clinical studies. Rice Bran Oil was negative in six RIPTs (maximum concentration tested was 8.0%). Rice Wax and Hydrogenated Rice Bran Wax were patch tested and produced "almost no acute primary irritation" in 27 subjects.* ***Extracts:*** *Rice Bran Extract functions as a biological additive and was used in five formulations, Rice Extract functions as a biological additive and was in four formulations. Rice Extract reduced the cytotoxicity of sodium chloride in male rats. Little safety test data were available.* ***Bran, Starch and Powder:*** *Rice Bran functions as an abrasive and bulking agent and was used in one formulation, Rice Starch functions as a skin conditioning agent - occlusive and was used in 64 formulations; Rice Germ Powder functions as an abrasive and was reported in one use for exfoliating purposes. Oral-dose carcinogenicity studies done on components of Rice Bran, phytic acid and γ-oryzanol were negative. Rice Bran did not have an anti-carcinogenic effect on DMH-induced large bowel tumors. In co-carcinogenicity studies done on Rice Bran Oil and Rice Bran-derived hemicellulose and saccharide, significant tumor inhibition was observed; γ-oryzanol did not inhibit the development of neoplasms. A decrease in cutaneous lesions in atopic dermatitis patients was reported following bathing with a Rice Bran preparation.* ***Proteins:*** *Hydrolyzed Bran Protein and Hydrolyzed Rice Protein function as conditioning agents (hair or skin). No uses were reported. Isolated cases of allergy to raw rice have been reported. The CIR Expert Panel concluded that the available data were sufficient to support the safety of the germ powder, starch, oils, fatty acids, and waxes as used in cosmetic products. The available data, however, could not be extended to extracts or the proteins derived from rice. For the extracts, additional data needs included: 1) UV absorption and, if there is significant absorption, then a dermal phototoxicity/photosensitization study will be needed; 2) dermal irritation and sensitization at concentration of use; and 3) ocular irritation, if available. For the bran, additional data needs included: 1) concentration of use; 2) contaminants (pending results, additional studies may be needed); 3) UV*

absorption and, if there is significant absorption, then a dermal phototoxicity/photosensitization study will be needed; 4) dermal irritation and sensitization at concentration of use; and 5) ocular irritation, if available. For the proteins, additional data needs included: 1) method of extraction; 2) UV adsorption and, if there is significant absorption, then a dermal phototoxicity/photosensitization study will be needed; 3) dermal irritation and sensitization at concentration of use; and 4) ocular irritation, if available. The Panel noted that none of these rice derived ingredients as used in products should not contain significant levels of pesticide residues or heavy metals.

INTRODUCTION

The original final report on the safety assessment of rice-derived ingredients found only Oryza Sativa (Rice) Germ Powder and Oryza Sativa (Rice) Starch safe as used in cosmetic formulations. This amended final report includes additional data supporting the safety of rice-derived oils, fatty acids, and waxes.

This report includes a compilation of data on **Oryza Sativa (Rice) Bran Oil** [CAS Nos. 68553-81-1 and 84696-37-7], **Oryza Sativa (Rice) Germ Oil, Rice Bran Acid** [CAS No. 93165-33-4], **Oryza Sativa (Rice) Wax** [CAS No. 8016-60-2], **Hydrogenated Rice Bran Wax, Oryza Sativa (Rice) Extract** [CAS No. 90106-37-9], **Oryza Sativa (Rice) Bran Extract, Oryza Sativa (Rice) Germ Powder, Oryza Sativa (Rice) Starch** [CAS No. 9005-25-8 -generic for all starches], **Oryza Sativa (Rice) Bran, Hydrolyzed Rice Bran Protein**, and **Hydrolyzed Rice Protein**. All ingredients are derived from rice, *Oryza sativa*. For brevity, the botanical name will be largely omitted from the text, but is included in the headings to remind the reader of the complete names.

The CIR Expert Panel considered the available data on the above rice-derived ingredients, but also reviewed data from earlier safety assessments of plant oils, acids, and starches, as well as Aluminum Starch Octenylsuccinate and Tocopherol. Those earlier findings included: Wheat Starch was found safe in the present practices of use and concentration (Elder, 1980); Cotton (Gossypium) Seed Oil, Cottonseed Acid, and Hydrogenated Cotton Seed Oil were found safe as used, provided that established limits on gossypol, heavy metals, and pesticide concentrations are not exceeded (CIR, 1998); Tocopherol was found safe as used (CIR 1999a); Safflower Oil was found safe in the present practices of use (Elder, 1985); and Aluminum Starch Octenylsuccinate was found safe as used provided that established limits on heavy metals are not exceeded (CIR, 1999b)

CHEMISTRY

Definition

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil is the oil expressed from rice *Oryza sativa* bran (Wenninger et al., 2000)

Oryza Sativa (Rice) Germ Oil is the oil obtained by the expression of rice germ *Oryza sativa* (Wenninger et al., 2000).

Rice Bran Acid (CAS No. 93165-33-4) is a mixture of fatty acids derived from Rice Bran (Oryza Sativa) Oil (q.v.) (Wenninger et al., 2000).

Oryza Sativa (Rice) Wax is a wax obtained from rice bran, *Oryza sativa* and Hydrogenated Rice Bran Wax is the end product of controlled hydrogenation of Rice Bran Wax (q.v.) (Wenninger et al., 2000).

Extracts

Oryza Sativa (Rice) Bran Extract is an extract of the bran of rice, *Oryza sativa* (Wenninger et al., 2000).

Oryza Sativa (Rice) Extract is an extract of the grains of rice, *Oryza sativa* (Wenninger et al., 2000).

Bran, Starch and Powder

Oryza Sativa (Rice) Bran is the broken hulls of rice *Oryza sativa* (Wenninger et al., 2000). Rice Bran is a by-product of rice milling obtained during polishing of rice. It is the part between the paddy husk and endosperm. Bran consists of 15-20% oil (Rukmini, 1988).

Starch, General - Informatics (1974) states that plant starch is an polymer which consists of monomeric units of D-anhydroglucose. The predominant linkage is 1,4-alphaglucosidic bonds. There are two basic starch polymers, amylose and amylopectin. They are both composed of anhydroglucose units but differ in the way they are linked and shaped.

Amylose is a linear polymer which contains 1,4-alphaglucose bonds between units. Each unit consists of one primary and two secondary hydroxyl groups except the terminal unit. It has reducing and non-reducing ends which contain varying numbers of hydroxyl units and an aldehydic reducing group.

Amylopectin is a highly branched structure and each branch contains 15-25 anhydroglucose units connected by 1,4 alpha linkages. Branches are connected by linkages attaching carbon 1 of the anhydroglucose unit at the beginning of the branch to carbon 6. Amylopectin is usually larger in size than amylose (Informatics, 1974).

Pasapane et al. (1999) reported 17% amylose and 83% amylopectin in Rice Starches. Starches can be physically and/or chemically modified for a specific need. Table 1 identifies the chemical modifications used in industry.

Oryza Sativa (Rice) Starch is a starch obtained from the endosperm of rice, *Oryza sativa* (CTFA, 1999b; Wenninger et al., 2000). Starches are a GRAS (generally recognized as safe) food ingredient (Informatics, 1974).

White rice, resulting from a process of milling called scouring or whitening of brown rice, is the endosperm of the kernel and is composed primarily of starch (90%) (Mazzo, 1998).

Oryza Sativa (Rice) Germ Powder is the powder derived from the rice germ, *Oryza sativa* (Wenninger et al., 2000).

Proteins

Hydrolyzed Rice Bran Protein is the hydrolysate of rice bran protein derived from acid, enzyme, or other method of hydrolysis (Wenninger et al., 2000).

Hydrolyzed Rice Protein is the hydrolysate of rice protein derived by acid, enzyme or other method of hydrolysis (Wenninger et al., 2000).

Table 1. Chemical Modifications of Starch (Pasapane et al., 1999)

General Type of Modification	Specific Modification	Description
Depolymerization	acid-hydrolysis	a strong acid is added to a granular starch slurried in water, and heat is added to degrade the starch
Depolymerization	oxidation	an oxidizing agent is added to a granular starch slurried in water, and heat is added to degrade the starch
Depolymerization	enzymatic action	enzymes attack and break down the starch molecule at very specific chemical links
Depolymerization	heating	dried, acidified starch is heated - also know as starch roasting
Addition of monofunctional substituent groups	hydrophobic	hydrophobic moieties are attached to the starch backbone
Addition of monofunctional substituent groups	cross-linking	the starch is treated to produce chemical cross-links, providing higher, more stable viscosities
Addition of monofunctional substituent groups	hydroxypropylation	β-hydroxypropyl groups are attached to the starch
Addition of monofunctional substituent groups	anionic/cationic groups	anionic and cationic groups can be added for particular functional attributes

Chemical Composition and Chemical/physical Properties

γ-Oryzanol

γ-Oryzanol (CAS No. 11042-64-1) is a phytosterol derived from Rice Bran (Hiraga et al., 1993) or Rice Bran Oil (Kubota and Sekine (1978). Where data are available on γ-Oryzanol in each section of this report, those data will be presented first. Kubota and Sekine (1978) patented an extraction technique from Rice Bran Oil and Rogers et al. (1993) reported an HPLC detection technique. Crude rice bran oil contains about 2% or more of γ-Oryzanol, a group of ferulate esters of triterpene alcohols and phytoesters.

McCaskill and Zhang (1999) stated that the high antioxidant properties of γ-Oryzanol are widely recognized and its ability to reduce plasma cholesterol, reduce cholesterol absorption and decrease early atherosclerosis, inhibit platelet aggregation, and increase fecal bile acid excretion have been studied. γ-Oryzanol has been used to treat nerve imbalance and disorders of menopause, although no food additive determinations have been made by the FDA (McCaskill and Zhang, 1999).

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil -Typical compositions of finished Rice Bran Oil samples are shown in Tables 2 and 3.

Table 2: Composition of Rice Bran Oil (Rukmini and Raghuram, 1991)

Fatty Acid		Percentage
Unsaturated Fatty Acids		
	oleic acid	38.4%
	linoleic acid	34.4%
	linolenic acid	2.2%
Saturated Fatty Acids:		
	palmitic acid	21.5%
	stearic acid	2.9%
Unsaponifiable Fraction (4.2%)		
	tocopherols	81.3 mg%
	γ-oryzanol	1.6%
	squalene	320 mg%

Table 3. Rice Bran Oil Fatty Acid Composition (Mccaskill and Zhang, 1999)

Fatty Acid	Percentage
Myristic	0.25%
Palmitic	21.5%
Stearic	2.9%
Oleic	38.4%
Linoleic	34.4%
Linolenic	2.2%
Arachidic	0.5%
Behenic	0.2%

Crude Rice Bran Oil is described as non-edible due to high free fatty acids and unsaponifiables. The unsaponifiable fraction also contains, "appreciable amounts" of waxes (Rukmini, 1988).

Tsuno Rice, Fine Chemicals Co., Ltd. (2000) provided the specifications and composition for Rice Bran Oil and Germ Oil (PRO-15) shown in Table 4.

Table 4. Rice Bran Oil and Germ Oil: Specifications and Composition (Tsuno Rice, Fine Chemicals Co., Ltd., 2000)

Specifications	Rice Bran Oil	Rice Germ Oil
Acid Value	0.1 max	0.2 max
Unsaponifiable Matter	3.5% max	6% max
Peroxide Value	2 meq/kg max	5 meq/kg max
Color (Lovibond 133.4 mm)	red 3.5 max; yellow 30 max	red 6.0 max; yellow 40 max
Specific Gravity	0.916-0.922	0.913-0.922
gamma-oryzanol	0.4%	1-1.5%

Composition	Rice Bran Oil	Rice Germ Oil
Cholesterol	0 g	0 g
Oleic Acid	42 g	42 g
Linoleic Acid	37 g	37 g
Phytosterol	900 mg	1600 mg
Tocopherol	30 mg	30 mg

Tocotrienol	25 mg	25 mg

Among the many sterols present in the unsaponifiable fraction of Rice Bran Oil, oryzanols and tocotrienols have been intensively studied. γ-Oryzanol was identified on the previous page and published studies concerning it are cited throughout this report. Tocotrienols are powerful antioxidants that belong to the vitamin E family. There are at least four forms known and are similar to the tocopherols in chemical structure. Rice Bran Oil is the only readily available oil, other than palm oil, that contains significant levels of tocotrienols (~1000 ppm). A significant portion of the tocotrienols are stripped away with distillate when the oil is deodorized. Tocotrienols may be recovered from the distillate by further fractionation techniques and added back to the oil (McCaskill and Zhang, 1999).

Oryza Sativa (Rice) Germ Oil

An oil registered in Japan as "rice germ oil" is a mixture of Rice Bran Oil and Rice Germ Oil (Ichimaru Pharcos Co., 1998). It contains 1.0 - 1.5% oryzanol. Studies using this mixture appear under the "rice germ oil" heading in this report.

Saker et al. (1986) evaluated some chemical components in Rice Germ Oil. The percentage of fat present in Rice Germ was 20.80 on dry basis respectively. The fatty acid composition is presented in Table 5. The saturated and unsaturated fatty acid contents of Rice Germ Oil were 27.19 and 71.22%, respectively. The percentage of unsaponifiable matter, phospholipids, and sterols were 4.51, 2.43, and 1.98%, respectively. The carotenoid content of Rice Germ Oil was 0.65 mg/g and the vitamin E content was 0.067%. Vitamins A, D, and K were absent.

Oryza Sativa (Rice) Wax and Hydrogenated Rice Bran Wax

Buffa (1976) reported two processing techniques for obtaining the two Waxes from Rice Bran Oil. In both techniques, Rice Bran Oil is processed (removal of gumming materials, de-acidation, and dewaxed) to obtain a solid oil-and-fat fraction (crude wax). In the first technique, the wax is extracted from this fraction with solvent and then bleached and refined, thereby creating Oryza Sativa (Rice) Wax. In the second technique, the crude wax is hydrogenated and then bleached, thereby creating Hydrogenated Rice Bran Wax. Physical properties of the waxes are in Table 6.

Table 5. Fatty Acid Composition of Rice Germ Oil (Saker et al., 1986)

Fatty Acids	% in Rice Germ Oil
Myristic	6.92
Palmitic	9.28
Stearic	7.91
Arachidic	3.08
Palmitoleic	4.41
Oleic	17.81
Linoleic	16.22
Linolenic	15.56
Arachidonic	5.48
Arachidotrienoic	5.21

Buffa (1976) also detailed the fatty acid and alcohol composition of the two waxes. These data are presented in Table 7a. (fatty acid) and 7b. (alcohol).

Extracts

Oryza Sativa (Rice) Extract

The specifications from one manufacturer identifies Rice Extract as containing 10-25% extract, > 75% sunflower seed oil (solvent used for extraction), and 0.15% of the preservative DL-α-tocopherol. The ingredient contains proteins, amino acids, peptides, flavonoids, lipids, mineral substances, vitamins, carbohydrates, and starch. It is soluble in oil soluble products, mineral oil, fatty oils (Grau Aromatics GmbH & Co., 1998). Physical properties are listed in Table 6.

Bran, Starch and Powder

Oryza Sativa (Rice) Bran

Rice Bran contains "a considerable amount of lipids", and some biologically active substances such as inositol, γ-oryzanol, and phytic acid (Fujiwaki and Furusho, 1992).

Table 6. Physical Properties of Rice Bran Oil and Rice Germ Oil Mixture, Rice Wax, Hydrogenated Rice Bran Wax, Rice Starch, Rice Extract, and Rice Bran Extract

Property	Rice Bran Oil and Rice Germ Oil mixture (Ichimaru Pharcos Co., 1994)	Rice Wax (Buffa, 1976)	Hydrogenated Rice Bran Wax (Buffa, 1976)	Rice Starch (CTFA, 1999b)	Rice Extract (Grau Aromatics GmbH & Co., 1998)	Rice Bran Extract (CTFA, 1999d)
Appearance	light yellow oil	white flakes	light yellow granules		clear, yellowish liquid	
Melting Point		79-83°C	70-77°C			
Specific Gravity	0.913 - 0.923	0.932-0.945	0.912-0.927	0.950		1.02-1.15
Saponification Value	180- 195	75-88	130-160			
Iodine Value	92 - 115	5 max	10 max			
Refractive Index	1.470 - 1.475	1.478-1.482	1.471-1.474	1.5045	1.465 -1.485	1.3860-1.5000
Density					0.910 - 0.930	
Solubility						soluble in any proportion in water
Heavy Metals Arsenic	10 ppm max 1 ppm max arsenic			10 ppm max (Pb)	1 ppm max ----------	
pH						4.0-6.5
Maximum Absorption				292 nm		
Plant Part Used				endosperm		bran

Table 7a. Fatty Acid Composition of Oryza Sativa (Rice) Wax and Hydrogenated Rice Bran Wax (Buffa, 1976)

Acid	Carbon No.	Rice Wax (%)	Hydrogenated Rice Wax (%)
Myristic Acid	C_{14}		.32
Palmitic Acid	C_{16}	3.28	18.94
Stearic Acid	C_{18}	.32	60.55
Oleic Acid	$C_{18}F_1$	.32	
Arachidic Acid	C_{20}	.69	1.35
Behenic Acid	C_{22}	16.24	3.64
Lignoceric Acid	C_{24}	42.62	9.26
Cerotic Acid	C_{26}	2.01	0.46
Montanic Acid	C_{28}	1.17	
Melissic Acid	C_{30}	2.78	
Lacceroic Acid	C_{32}	1.33	
Tetratriacontanoic Acid	C_{34}	1.1	
Hexatriacontanoic Acid	C_{36}	.56	

Table 7b. Fatty Alcohol Composition of Oryza Sativa (Rice) Wax and Hydrogenated Rice Bran Wax (Buffa, 1976)

Alcohol	Carbon No.	Rice Wax (%)	Hydrogenated Rice Wax (%)
Behenyl Alcohol	$C_{22}OH$	.38	
Lignoceryl Alcohol	$C_{24}OH$	3.21	.92
Ceryl Alcohol	$C_{26}OH$	2.93	1.17
Octacosyl Alcohol	$C_{28}OH$	5.59	1.3
Myricyl Alcohol	$C_{30}OH$	8.35	1.63
Lacceryl Alcohol	$C_{32}OH$	4.64	0.42
Tetratriacontyl Alcohol	$C_{34}OH$	2.22	
Hexatriacontyl Alcohol	$C_{36}OH$	.5	

Proteins

Hydrolyzed Rice Protein

Hydrolyzed Rice Protein is extracted from rice grains and then is enzymatically digested (CTFA, 1999a). A standard profile of Hydrolyzed Rice Protein components is shown in Table 8.

Table 8. Component Profile of Hydrolyzed Rice Protein (CTFA, 1999a)

Component	Percentage
Protein	Approximately 60% by weight
Carbohydrates	24%
Moisture	<6%
Fat	0.4%
Ash	7%
Sodium	2.4%

Method of Manufacture

Mazzo (1998) describes the commercial market for three forms of rice: rough, brown, and white. Rough rice is the harvested unshelled rice, while brown rice is rice from which the hull has been removed by shelling or hulling. White rice, milled rice, is rice in which all or most of the bran has been removed by some operation of milling called scouring or whitening. Usually the milling is followed by the operation of polishing in which remaining traces of bran are removed from the kernel.

Rice Bran is extracted for oil and for its protein. Commercial rice bran contains 11.5-17.2% protein, 12.8-29.6% fat, 6.2-31.5% fiber, and 8-17.7% ash, depending on processing. The amount of starch in the bran ranges from 10-55%, depending on the degree of milling. Phosphorus, primarily in the form of phytates, is the major mineral constituent of rice bran. Potassium, magnesium, and silicon are also present at high levels.

An overview of both commercial and theoretical rice bran processing and utilization are shown in Figure 1. The process of milling abrades the external cell layers (the bran) down to the endosperm, thoroughly mixing the bran material. Native lipase enzymes come in contact with the oil in the aleurone and sub-aleurone layers, causing rapid hydrolysis of the oil fraction within raw bran; this results in a rapid increase in free fatty acids and glycerol. This enzymatic deterioration, or lipolysis of the oil fraction within the raw bran, is known as hydrolytic degradation.

Heating bran in the presence of moisture permanently denatures lipolytic enzymes and destroys lipolytic microbes. Stabilization processes preserving rice bran from hydrolytic degradation are accomplished by three processes: retained-moisture heating, added-moisture heating, and dry heating at atmospheric pressure. Low levels of trysin inhibitors and hemagglutinin from the germ are present in raw rice bran and are destroyed under conditions that denature lipolytic enzymes.

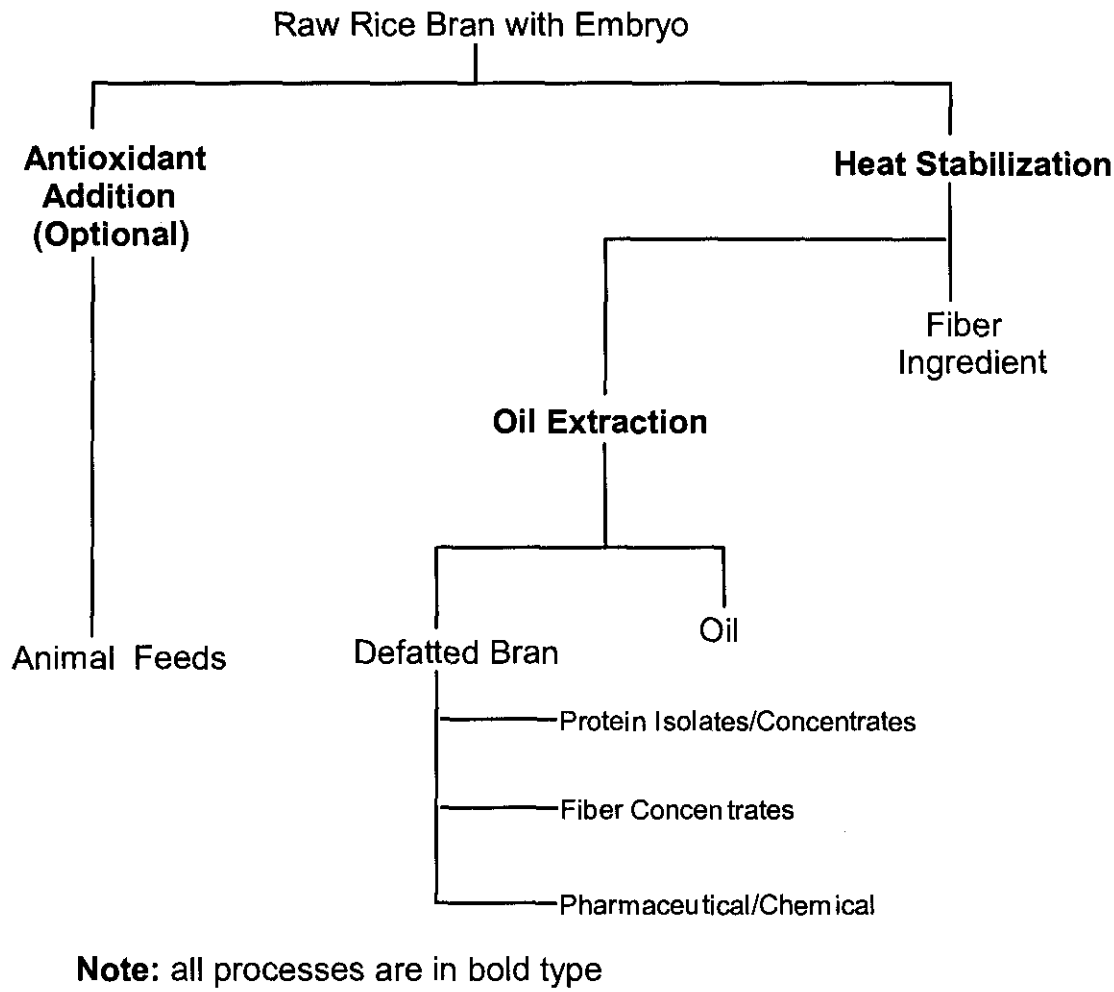

Figure 1. Processing and Utilization of Rice Bran (Mazzo, 1998)

The Rice Bran (with germ) fraction contains the majority of the oil in the kernel. The oil content of clean rice bran is 20-22%, which is similar to that of soybeans and cottonseed. Once milled, Rice Bran Oil is exposed to lipases in the bran, which results in its rapid breakdown to free fatty acids at an initial rate of 5-7% by weight of the oil per day. Due to the rapid increase in free fatty acids, either refining of the edible oil or rice bran stabilization by enzyme inactivation must occur as soon as possible after milling to prevent excessive oil refining loss. The yield of refined Rice Bran Oil depends on the age and storage conditions of the rice, milling practices, bran stabilization, conditions used for extraction, and the method of refining the oil. Extraction can occur at high or low temperatures. Many organic solvents can be used for Rice Bran Oil extraction, with the most popular being *n*-hexane. Hexane extraction at about 60°C results in the inclusion of most of the gums and waxes, which yields a greater quantity of crude oil, but only an 80% yield of refined oil. The gums and waxes are then removed. Conversely, low-temperature extraction at about 18°C removes neutral oil, with minimal quantities of gums and waxes, and may yield 98% refined oil.

The two types of refining used with Rice Bran Oil are alkali and physical. Alkali refining works well

with oils that contain relatively high amounts of free fatty acids, but results in a greater loss of neutral oil. Physical refining depends on molecular distillation from a thin film, and refining losses can approach the actual free fatty acid content. Physical refining is usually followed by a light alkali refining to remove the last traces of free fatty acids. Discoloration due to high levels of free fatty acids and wax are the problems most often encountered in refining Rice Bran Oil. Timely bran stabilization and careful control of temperatures during extraction and refining can greatly reduce these problems. Refined oil can be bleached with activated bleaching clay just as any other vegetable oil. High-temperature hexane extraction may result in 3-4% wax in the crude oil. These waxes can be isolated and purified by crystallizing and precipitating at low temperatures. The waxes may be centrifuged or filtered off and then washed with acetone or ethanol to remove residual oil (Mazzo, 1998).

A manufacturer of Rice Waxes reported that the waxes from rice are present as an impurity in Rice Oil. The Rice Oil is manufactured from Rice Bran produced at the same stage when the rice grains are milled. The product obtained from Rice Oil by solvent extraction is crude rice wax. This is refined to obtain refined rice wax. The product Ricebran Wax (S-100) is obtained from Rice Bran by the process of extraction and separation. The product R-100 is obtained by hydrogenating S-100 and retaining a suitable concentration of Rice Oil in the Hydrogenated Rice Wax (Yokozeki Oil & Fat Industries Co., Ltd., 2000).

Contaminants

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil

Rice Bran Oil is used extensively in cooking in Asian countries. The published literature contains numerous articles concerning two incidences of polychlorinated biphenyl (PCB)-contamination in Rice Bran Oil. Exposure was documented in 1968 in western Japan, and in 1979 in central Taiwan (Chen et al., 1984; IARC, 1987; Schantz, 1996). PCB-intake was estimated at 0.7-1.84 g/person (Hsu et al., 1984). The oils had also been contaminated with polychlorinated quaterphenyls (PCQs) and polychlorinated dibenzofurans (PCDs) and some investigators considered PCDs to be the most important etiologic agents for the observed symptoms and signs of poisoning (Kunita et al., 1984; Masuda and Yoshimura, 1984).

Extracts

Oryza Sativa (Rice) Bran Extract

Information provided by suppliers gave the following contaminants and limits: 1,4-dioxane (<50 ppm); benzene (<50 ppm); chloroform (<25 ppm); methylene chloride (<50 ppm); trichloroethylene (<50 ppm); heavy metals as lead (<20 ppm); arsenic (<3 ppm); iron (<100 ppm); and microbial plate count (<100 organisms/gm) (CTFA, 1999d).

Concentrations of other components of the raw material was reported as: 97-98.8% solvent (water/propylene glycol, water/butylene glycol, water/glycerin, safflower oil) and 1% preservative (CTFA, 1999d).

Bran, Starch and Powder

Oryza Sativa (Rice) Starch

The Food Chemicals Codex limits of impurities on unmodified food starches are; not more than 0.002% heavy metals, not more than 1mg/kg lead, and not more than 0.005% sulfur dioxide (National Academy of Sciences, 1996).

UV Absorption

Several patents for sunscreen formulations describe use of rice bran-derived ingredients. One patent by Loo (1976) reported that Rice Bran Oil applied either undiluted or in a topical formulation was an, "effective sunscreen" against exposure to UV radiation at 295-315 nm. The absorption differential (transmittancy of tanning rays 315-365 nm/transmittancy of burning rays 295-315 nm) was, "many times higher" than that of other oils or sunscreen formulations. It "absorb(ed) UV rays in the burning region to a much greater extent" than other oils or commercial preparations.

Typically, formulations contain 5 or 6% Rice Bran Oil (Loo, 1980; Potter and Pugliese, 1994; 1995), or 5 parts (by weight) Rice Wax (Yoshida et al., 1990).

A patent by Ishibashi (1994) reported that a skin oil containing 3% γ-oryzanol had a SPF of 3.

Oryzanol had absorption maxima at 231, 291, and 315 nm (Ichimaru Pharcos Co., unknown date). A mixture of Rice Bran Oil and Rice Germ Oil had an absorption maximum at 315 nm (Ichimaru Pharcos Co., 1994). Phototoxicity studies on the oil mixture and oryzanol appear in the Phototoxicity section of this report.

USE

Cosmetic

The functions of the various rice bran-derived ingredients in cosmetic formulations are listed in Table 9. In addition, one manufacturer reported Rice Germ Powder use as an exfoliant (CTFA, 1999c).

As shown in Table 10, industry reports to FDA listed the oil ingredients in 40 formulations, 35 of which were Rice Bran Oil. Rice Wax (identified as rice bran wax) had 6 uses, and Hydrogenated Rice Bran Wax had 11 uses. Rice Bran Extract (and its lipid fraction) was used in 5 formulations, Rice Starch in 48 formulations, and Rice Bran (identified as rice hulls) was used in one formulation, (FDA, 2001). Hydrolyzed Rice Protein was reported to be used at 0.5-1.0% in skin care products and 0.1 - 2.0% in hair care products, but no specific product types were given (CTFA, 1999a)..

Table 9. Cosmetic Functions of Rice Bran-derived Ingredients (CTFA 1999a; Wenninger et al., 2000)

Ingredient	Chemical Class	Function
		oils, fatty acids, & waxes
Oryza Sativa (Rice) Bran Oil	fats and oil	skin-conditioning agent -occlusive
Oryza Sativa (Rice) Germ Oil	fats and oils	skin-conditioning agent -occlusive
Rice Bran Acid	fatty acids	surfactant -cleansing agent
Oryza Sativa (Rice) Wax	waxes	skin-conditioning agent -occlusive
Hydrogenated Rice Bran Wax	waxes	binder
		skin-conditioning agent -occlusive
		viscosity increasing agent -nonaqueous
		extracts
Oryza Sativa (Rice) Bran Extract	biological products	not reported
Oryza Sativa (Rice) Extract	biological products	hair-conditioning agent
		skin-conditioning agent-miscellaneous
		bran, starch & powder
Oryza Sativa (Rice) Bran	biological products	abrasive; bulking agent
Oryza Sativa (Rice) Starch	carbohydrates	absorbent; bulking agent
Oryza Sativa (Rice) Germ Powder	biological products	abrasive
		proteins
Hydrolyzed Rice Bran Protein	protein derivatives	hair conditioning agent
		skin-conditioning agent -miscellaneous
Hydrolyzed Rice Protein	protein derivatives	hair conditioning agent
		skin conditioning agent -miscellaneous
		antistatic agent

Table 10. Frequency and Concentration of Use

Product Category (Total formulations in category) (FDA, 2001)	Formulations in Category Containing Ingredient (FDA, 2001)	Current Concentration of Use (CTFA, 2000)
Rice Bran Oil		
Bath Oils, Tablets, and Salts (140)	1	1-39%
Other Bath Preparations (159)	-	1%
Eyebrow Pencils (91)	-	0.1%
Eye Lotion (18)	-	1%
Mascara (187)	-	0.1%
Other Makeup Preparations (120)	-	0.5%
Hair Conditioners (630)	2	0.3%
Other Hair Preparations (276)	1	-
Foundations (319)	1	0.5%
Lipstick (790)	-	0.1 - 1%
Makeup Bases (132)	-	3%
Other Manicuring Preparations (57)	2	-
Bath Soaps and Detergents (385)	-	1%
Other Shaving Preparation Products (60)	-	1%
Skin Cleansing (733)	2	0.5 - 1%
Face and Neck Skin Care (excl shaving) (304)	5	0.3 - 3%
Body and Hand Skin Care (excl shaving) (827)	2	3 - 4%
Moisturizing Creams, Lotions, Powders, and Sprays (881)	10	8%
Night Creams (200)	-	0.3%
Paste Masks (mud packs) (269)	2	0.2%
Other Skin Care Preparations (715)	5	-
Suntan Gels, Creams, and Liquids (131)	2	3%
2001 total for Rice Bran Oil	**35**	
Rice Germ Oil		
Cleansing (733)	1	-
Face and Neck (excl shaving) (304)	1	-
Other Skin Care Preparations (61)	1	-
Body and Hand Skin Care (excl shaving) (796)	2	0.1%
2001 total for Rice Germ Oil	**5**	
Rice (Bran) Wax		
Foundations (319)	1	
Lipstick (942)	2	-
Face and Neck skin care (excl. shaving) (304)	2	-
Suntan Gels, Creams, and Liquids (131)	1	-
1998 total for Rice (Bran) Wax	**6**	

Table 10. (Continued) Frequency and Concentration of Use

Product Category (Total formulations in category) (FDA, 2001)	Formulations in Category Containing Ingredient (FDA, 2001)	Current Concentration of Use (CTFA, 2000)
Hydrogenated Rice Bran Wax		
Eyebrow Pencil (99)	3	-
Eyeliner (533)	1	-
Other Eye Makeup Preparations (151)	3	-
Lipstick (942)	1	-
Other Makeup Preparations (186)	3	-
2001 total for Hydrogenated Rice Bran Wax	**11**	
Rice Extract		
Cleansing (733)	1	-
Other Skin Care Preparations (276)	2	-
Body and Hair Skin Care (715))	1	-
2001 total for Rice Extract	**4**	
Rice Bran Extract		
Tonics, Dressings, and Other Hair Grooming Aids (577)	2	-
Other Hair Preparations (276)	1	-
Moisturizing (881)	1	-
Other Skin Care Preparations (715)	1	-
2001 total for Rice Bran Extract	**5**	
Rice Bran Extract, lipid fraction*		
Body and Hand Skin Care (excl shaving) (827)	1	-
2001 total for Rice Bran Extract, lipid fraction	**1**	
Rice Starch		
Bath Oils, Tablets, and Salts (124)	-	97%
Eyeliner (514)	-	8%
Eye Shadow (551)	1	3%
Powders (247)	-	6%
Hair Conditioners (630)	-	4%
Mascara (187)	5	4%
Other Eye Makeup Preparations (151)	3	-
Other Hair Preparations (276)	1	-
Blushers (all types) (243)	2	9%
Face Powders (301)	4	1%
Foundations (319)	2	-
Bath Soaps and Detergents (405)	1	-
Face and Neck skin care (excl. shaving) (304)	5	2%
Body and Hand skin care (excl. shaving) (827)	7	-
Moisturizing (881)	1	4%

Table 10. (Continued) Frequency and Concentration of Use

Product Category (Total formulations in category) (FDA, 2001)	Formulations in Category Containing Ingredient (FDA, 2001)	Current Concentration of Use (CTFA, 2000)
Rice Starch (continued)		
Night Creams (200)	1	2%
Paste Masks (mud packs) (269)	8	-
Skin Fresheners (181)	1	-
Other Skin Care Preparations (715)	6	-
2001 total for Rice Starch	**48**	
Rice Bran (identified as rice hulls)		
Other Personal Cleanliness Products (307)	1	-
2001 total for Rice Bran	**1**	

* while reported to be used, this ingredients is not identified as a cosmetic ingredient (Wenninger et al., 2000)

Current concentration of use data provided by industry list concentrations of use for several other product types (CTFA, 2000). These data are also shown in Table 10.

One supplier noted use of Rice Extract at 1-10% in cosmetics, but did not identify product type(s) (Grau Aromatics, 1998). Another company used Rice Bran Extract at low (trace) levels (CTFA, 1998). Current data were not found for the other ingredients. Historical data stated that 28 of the 33 reported cosmetic uses of Rice Bran Oil were at ≤ 1%; another two entries were in the 1-5% category and one entry each was in the 5-10%, 10-25% and 25-50% categories and Rice Wax (identified as rice bran wax) was used in lipsticks at 0.1-5% (FDA, 1984).

International

The CTFA *International Cosmetic Ingredient Dictionary and Handbook* notes that the ingredients, Oryza Sativa (Rice) Bran, Oryza Sativa (Rice) Bran Extract, Oryza Sativa (Rice) Bran Oil, Oryza Sativa (Rice) Extract, Oryza Sativa (Rice) Germ Oil, Oryza Sativa (Rice) Germ Powder, Oryza Sativa (Rice) Starch and Oryza Sativa (Rice) Wax will all be labeled "Oryza Sativa" in the European Union when regulations for ingredient labeling under the 6th Amendment to the *EC Cosmetics Directive* go into effect (Wenninger et al., 2000).

There are no restrictions for the use of any of the Rice ingredients listed in this report in cosmetics in Japan according to the Ministry of Health, Labor, and Welfare (2000).

Non-cosmetic

General Food Uses

Oryza Sativa (Rice) is the staple food source for half of the world's population. It is non-allergenic, easily digested, and provides protein with higher nutritional quality than that in other cereal grains. The rice kernel is composed of a hull, caryopsis or brown rice, and the embryo. The hull comprises about 20% of the rice kernel, the bran and embyro about 8-12%, and the endosperm or milled rice (white rice) about 70-72% (Mazzo, 1998).

Per capita consumption of rice rose to 26.29 pounds in 1999 from 25.38 pounds in 1998. Direct food use (regular-milled, brown, parboiled, and precooked) accounted for 58% of rice sold in the United States. Food processing accounted for 25%, and beer brewing another 17%. All categories had an increase in consumption from 1998 to 1999. Direct food use rose 5%, processed foods went up 10%, and beer use rose 2.5% (USA Rice Federation, 1999a).

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil

Rice Bran Oil can be used in practically any application to replace other vegetable oils. Some of the uses include frying, margarine component, and coating to extend self-life of other food products (McCaskill and Zhang, 1999).

Oryza Sativa (Rice) Wax

Food uses for Rice Wax are mold release agent, brightener, coatings for chocolates, cakes, and tablets, treatment of vegetables and fruits, plasticizing material of chewing gum. Industrial uses include polish for cars, floors, and shoes, office ink, textile oiling agent, and resin lubricant (Yokozeki Oil and Fat Industries Co., Ltd., 2000).

Bran, Starch and Powder

Oryza Sativa (Rice) Bran

Rice Bran is the outermost layer on brown rice and gives it its color and nutty flavor. An excellent source of thiamin, niacin, vitamin B-6, iron, phosphorous, magnesium, potassium, and fiber, Rice Bran is an ingredient in cereals, mixes, and vitamin concentrates. The non-food grades are used to feed livestock (USA Rice Federation,1999b).

Oryza Sativa (Rice) Starch

Present only in the endosperm of the grain, Rice Starch makes up 90-93% of the milled rice dry weight. Native Rice Starch has many applications such as laundry-stiffening agent, paper and photographic paper powder, sugar coating in confectionery, and excipients in pharmaceuticals. Gelantinized Rice Starch is creamy and spreadable, good for custards and puddings. Waxy Rice Starch has excellent freeze-thaw stability and is used as a fat replacer in frozen dessets and gravies. Rice maltodextrins are commercially produced by hydrolyzing Rice Starch at high temperatures or with enzymes. These products serve as carriers for flavor and provide bulk in products such as frostings, soups, sauces, and salad dressings (Mazzo, 1998).

Miscellaneous

Oryza Sativa (Rice) Flour

Rice Flour is non-allergenic and is very valuable to persons allergic to gluten and wheat flour products. The flour is extruded to produce rice pasta, chips, and other snacks, as well as cereals (USA Rice Federation, 1999b).

Broken Kernels

Broken Kernels are used to make various products, including rice flour and pet foods (USA Rice Federation, 1999b).

GENERAL BIOLOGY

Skin Absorption

The Cosmetic Ingredient Review safety assessment of Wheat Germ Oil (Elder, 1980) described a report by Valette and Sorbrin (1963), to the effect that the rate of skin absorption was fastest for linseed oil and slowest for rice oil, with Wheat Germ Oil having an intermediate rate of absorption.

Hematologic Effects

Bran, Starch and Powder

Oryza Sativa (Rice) Bran

Takenaka and Itoyama (1993) reported a significant ($p < 0.05$) increase in the number of granular leukocytes and lymphocytes in rats given a 10% Rice Bran fiber diet for two weeks. A significant increase ($p < 0.01$) was also noted in rats that had received 10% hemicellulose (prepared from the fiber); 1 and 2% hemicellulose-diets produced changes comparable to the control diet of unaltered feed. The investigators considered the Rice Bran fiber hemicellulose to be promising in the management of leukopenia.

Protective Effects

γ-Oryzanol

γ-Oryzanol is reported to have a "strong affinity" for the skin; it covers it closely and has a suppressive effect on increases in keratin (Ueda et al., 1976).

A review by Wheeler and Garleb (1991) disputed the benefits of consuming γ-oryzanol (and other plant sterols) by athletes for anabolic purposes. It was noted that < 5% of orally consumed phytosterols are absorbed from the intestinal tract, with the majority being excreted in the feces. The reviewers noted that i.v. or s.c. administration of γ-oryzanol to rats has produced such catabolic events as: suppressed release of luteinizing hormone, reduced synthesis and release of growth hormone, and increased release of dopamine and norepinephrine in the brain.

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil

Jayaraj et al. (1986; 1987) reported that fresh Rice Bran Oil protects against gastric ulceration in rats, whereas stored oil is ulcerogenic. The protection was restored with the addition of cysteine to the stored oil. Lloris et al. (1991) reported significant reductions in the ulcer index ($p < 0.01$) and decreased H^+ concentrations in gastric juices ($p < 0.05$) of rats that had been pretreated with Rice Bran Oil prior to induction of stress ulcers. No changes were noted in the output volume of gastric juices or in gastric concentrations of histamine or pepsin. H^+ concentrations were similarly lower in Rice Bran Oil-treated rats following histamine stimulation, but no significant differences were noted following stimulation with two other stimulators of gastric secretion, betanechol or pentagastrin. The investigators noted that the oil contains a large percentage of unsaturated fatty acids that can act as precursors in Arachidonic Acid synthesis, which in turn is a precursor of prostaglandins. In addition, Rice Bran Oil contains anti-oxidants such as α-tocopherols that could have exerted a protective effect.

Extracts

Oryza Sativa (Rice) Extract

Furihata et al. (1996) reported decreased gastric mucosal damage and significantly reduced ($p < 0.01$) replicative DNA synthesis in male F344 rats that had received a concentrated commercial Rice Extract via gastric intubation 3 hours prior to administration of sodium chloride.

Bran, Starch and Powder

Oryza Sativa (Rice) Bran

Rice Bran fiber (10% in the feed) reduced bis(tri-n-butyltin)oxide (TBTO) -induced thymus atrophy in rats. A significant difference in relative thymus weights ($p < 0.01$) was noted in rats that had received rice bran fiber concurrent with TBTO exposure compared to rats that had received TBTO and basal diet. Further investigation established that hemicellulose was responsible for the reduction (Takenaka, 1992).

Tyrosinase Activity Inhibition

γ-Oryzanol

γ-Oryzanol was tested for its ability to inhibit the tyrosinase-tyrosine relationship in the "skin blackening phenomenon -abnormal melanin deposition". L-ascorbic acid, a known tyrosinase inhibitor, was used as the reference. A liquid containing 2% agar, 0.2% inhibitor, and 0.1% L-tyrosine was prepared and cooled to solidification. A 0.5% tyrosinase solution was added to the surface and the mixture was incubated. Blackening of the surface of the agar mass was observed. Oryzanol inhibited the formation of melanin (though it was weaker than L-ascorbic acid). The structure of the ferulic acid moiety in oryzanol was considered to resemble tyrosine thereby blocking enzymatic activity (Ichimaru Pharcos, unknown date).

Hypolipidemic Action

γ-Oryzanol

γ-Oryzanol is described as a drug used mainly for the treatment of hyperlipidaemia (Tamagawa et al., 1992a). In clinical studies, effects were noted following a typical dose of 300 mg/day p.o. for at least three months (Yoshino et al., 1989; Sasaki et al., 1990).

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil

Purushothama et al, (1995) reported comparatively lower concentrations of cholesterol (TC), triglycerides (TG) and phospholipids in rats that had received either 5 or 20% Rice Bran Oil in the diet compared to control rats that had received similar concentrations of Peanut Oil. A significant

increase ($p < 0.05$) in high density lipoproteins (HDL) was noted in rats that received 20% Rice Bran Oil compared to controls. Rice Bran Oil-fed rats also had lower low density lipoprotein (LDL) cholesterol and very low density lipoprotein cholesterol (VLDL) compared to controls. The investigators considered that feeding high doses of Rice Bran Oil to rats produced "no deleterious effect on growth or the blood lipid profile".

Similar results were reported earlier by Seetharamaiah and Chandrasekhara (1989). Serum total, free, esterified and (LDL+VLDL)-cholesterol concentrations were significantly lower in rats maintained on 10% Rice Bran Oil compared to control rats that had received Peanut Oil; hepatic lipids were lower in Rice Bran Oil-fed rats. HDL-cholesterol tended to be greater in rats of the Rice Bran Oil group. A further significant decrease in serum total cholesterol (but not in hepatic lipids) was noted when 0.5% oryzanol was added to the Rice Bran Oil diet. Oryzanol and other components of the unsaponifiable matter of Rice Bran Oil was considered responsible for the cholesterol lowering.

In clinical studies, a significant reduction ($p < 0.001$) in TC and TG was documented in twelve subjects with high TC, fifteen and thirty days after using Rice Bran Oil instead of their usual cooking oil. The response was greater in subjects with greater initial TC and TG values (Raghuram et al., 1989).

In reviews of the hypolipidemic action of Rice Bran Oil, Rukmini and Raghuram (1991) and Raghuram and Rukmini (1995) reported that the fatty acid content of Rice Bran Oil is similar to Peanut Oil. However, the unsaponifiable fraction of Rice Bran Oil contains more phytosterols, triterpene alcohols, tocopherols, and tocotrienols than do other oils. They reported that in animal studies, cycloartenol, a triterpene alcohol present in Rice Bran Oil, effectively lowered cholesterol and TG concentrations when compared to other edible oils. Data suggested that cycloartenol was absorbed and accumulated in the liver. Its structure is similar to cholesterol and the reviewers noted that it could compete for binding sites. Cycloartenol also inhibited cholesterol esterase activity thereby delaying release of cholesterol into the circulation. The reviewers considered that the hypocholesterolemic action of dietary fat depended primarily on the minor components of the unsaponifiable fraction and, to a lesser extent, on the fatty acid content of the oil.

Lichtenstein et al. (1994) reported that consumption of Rice Bran Oil-enriched diet by middle-aged and elderly subjects with moderately elevated concentrations of LDL-cholesterol, resulted in plasma lipid and apolipoprotein concentrations and predictive ratios of cardiovascular risks that were similar to those of more commonly used vegetable oils in the U.S. such as corn oil and canola oil. A greater than predicted reduction in plasma total cholesterol was noted with Rice Bran Oil treatment and was attributed to the unsaponifiable fraction.

Bran, Starch and Powder

Oryza Sativa (Rice) Bran

Sanders and Reddy (1992) reported that Rice Bran (without the fatty acid components of the oil) did not significantly alter body weight or plasma TC, LDL, HDL, apoprotein AI and B concentrations compared to wheat bran in 18 males with normal cholesterol concentrations. A significant decrease ($p < 0.05$) in plasma TG concentration was noted with 15 g/day Rice Bran compared to wheat bran.

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil

Rice Bran Oil had an oral LD_{50} of > 5 g/kg in white rats (Leberco Testing Inc., 1993a).

Oryza Sativa (Rice) Germ Oil

A mixture of Rice Bran Oil and Rice Germ Oil had an LD_{50} of > 40 ml/kg in mice (Ichimaru Pharcos Co., 1981a).

A group of 10 Sprague-Dawley rats (5 males, 5 females) were administered by oral intubation a single dose of 5 g/kg of body weight of Rice Germ Oil-K. The animals were observed for 14 days after administration. No clinical abnormalities were noted and no mortalities occurred. The animals were killed and no gross abnormalities were observed at necropsy. The LD_{50} was > 5 g/kg of body weight — Rice Germ Oil was not considered to be toxic (Celsis, 1999).

Oryza Sativa (Rice) Wax and Hydrogenated Rice Bran Wax

Rice Wax suspended in 25% gum arabic solution had an oral LD_{50} of > 24 g/kg in male mice (Nippon Bio-Test Laboratories, Inc., 1972).

Hydrogenated Rice Bran Wax (administered 50% in corn oil) had an oral LD_{50} of > 5 g/kg in white rats. Rats were killed 14 days after dosing and necropsied; one male rat had a dilated right kidney (Leberco Testing Inc., 1991a).

Consumer Product Testing Co. (1998f) conducted a study in which ten (5 male and 5 female) albino rats each received a single oral dose of Rice Bran Wax S-100 (Lot #W90305) at a dose of 5 g/kg bw. Animals were observed for pharmacological activity and drug toxicity 1, 3, 6, and 24 h after treatment, and daily thereafter for a total of 14 days. At the end of 14 days, the rats were killed and subjected to gross necropsy. The test article was used as a 12.5% suspension heated and cooled in corn oil.

No gross changes were observed in 9 of the rats. In one animal, two red nodules attached to fat adjacent to the bladder approximately 3 mm in diameter each and firm to the touch were observed. The LD_{50} > 5 g/kg. The test article was not orally toxic to rats (Consumer Product Testing Co., 1998f).

Chronic Oral Toxicity

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil

Following the food safety evaluation protocol of WHO/FDA/DGHS, Rukmini (1988) fed a group of 30 albino rats (Wistar strain, 15 each sex) a diet containing 10% edible-grade Rice Bran Oil, 20% protein, and adequate amounts of other nutrients. A control group received feed containing 10% Peanut Oil. After 100-120 days the rats were mated. Mating, gestation, lactation, and weaning were followed to obtain F_{1a} pups.

A week after weaning, F_0 parents were mated again to obtain mating pups F_{1b}. The procedure was continued until F_{3b} pups were obtained at which time all rats were killed.

Blood samples obtained prior to study termination were analyzed for TC and TG. The liver was analyzed for total lipids, TC and TG, and microscopic examination was done on the heart, lungs, kidneys, ovaries/testes, pancreas, and thymus.

Body weight gain, feed efficiency, fat absorption, nitrogen retention, and organ weights were comparable between Rice Bran Oil-fed rats and control rats. A hypocholesterolemic effect was noted in Rice Bran Oil-fed rats as indicated by the lipid profile (Rukmini, 1988).

Dermal Irritation and Sensitization

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil

Undiluted Rice Bran Oil was applied as a single occlusive patch to nine rabbits. Reactions were scored at 2 and 24 h after placement of the occlusive patch. The PII for the group was 0.0; the maximum possible score was 8 (CTFA, 1983).

A moisturizer containing 8.0% Rice Bran Oil applied as a single occlusive exposure to six rabbits had a PII of 1.67 (CTFA, 1987a).

Rice Bran Oil was tested in a Magnusson-Kligman maximization test. During induction groups of 10 shaved Dunkin-Hartley guinea pigs received two injections of each 50% Freund's Complete Adjuvant (FCA), 5% Rice Bran in propylene glycol, and 5% Rice Bran Oil in FCA (total of six injections). Controls were injected with FCA and propylene glycol. One week later, a topical booster was applied. Because preliminary testing established that 100% Rice Bran Oil did not produce irritation, 5% Sodium Lauryl Sulfate (SLS) in petrolatum was applied 24 h prior to the topical booster. Controls were pre-treated with SLS and petrolatum was applied during the booster phase. All guinea pigs were occlusively wrapped for 48 h. Two weeks later, guinea pigs were topically challenged with 24 h occlusive patches of 25% and 50% Rice Bran Oil. Challenge sites were graded 24 and 48 h after patch removal. No reactions were observed (CTFA, 1984).

Rice Bran Oil (0.5 ml) was applied to an abraded and intact hair-less site on the back of six New Zealand white rabbits. Sites were covered with a patch and the trunk of each rabbit was encased with an occlusive wrapping for 24 h of exposure. Sites were examined for erythema and edema using the Draize scoring scale at the time of

wrapping removal and 48 h later. Scores for the two observation times were averaged to calculate a primary irritation index (PII; scores > 5.0 indicated a primary dermal irritant). "Very slight to well-defined" erythema and "very slight" edema were observed, with a PII of 0.88 (Leberco Testing Inc., 1993b).

Oryza Sativa (Rice) Germ Oil

Following a modified-Draize method, a mixture of Rice Bran Oil and Rice Germ Oil (0.5 ml) was applied to abraded and intact sites on six female albino rabbits. The sites had been clipped free of hair. The sites were covered for 4 h of exposure and then any remaining test material was removed with ethanol. Sites were evaluated at 24 and 48 h. No reactions were observed (Ichimaru Pharcos Co., 1981b).

A skin contact allergy test was conducted using nine female Hartley guinea pigs. Six guinea pigs were sensitized. These six were injected (in the clipped neck) with an emulsion containing a mixture of Rice Bran Oil and Rice Germ Oil, sodium chloride solution, and Freund's Complete Adjuvant in a 1: 1: 2 volume. A 0.2 ml dose was divided and injected into four sites. One week later, the neck was again clipped of hair and 0.5 ml of the test material was applied. The site was covered with a polyethylene film for 48 h. Two weeks later, a 24 h patch containing Rice Germ Oil (0.1 ml) was applied to all nine guinea pigs. Sites were evaluated at the time of patch removal, and 24 and 48 h later. No changes were observed (Ichimaru Pharcos Co., 1979a).

Primary dermal irritation was assessed using six New Zealand white rabbits by applying single doses of 0.5 ml Rice Germ Oil-K to two test sites. Both sites were located on each side of the animals vertebral column mid-dorsally. The site on the left was maintained intact and the the site on the right was abraded with longitudinal epidermal incisions, sufficiently deep to penetrate the stratum corneum. The sites were then completely encased in an impermeable occlusive wrapping. The wrapping and test article were removed after 24 h following application. Erythema and edema were scored using the Draize skin scoring scale. The test material produced a very-slight to well-defined erythema at the 24 h observation. No edema was noted. The readings were averaged to determine the primary irritation index. The primary irritation score for this test substance was 0.75 — Rice Germ Oil was not classified as a primary dermal irritant (Celsis, 1999).

Oryza Sativa (Rice) Wax and Hydrogenated Rice Bran Wax

Using the above protocol, Rice Wax (0.5 g) was applied to intact and abraded sites on six white rabbits. Sites were covered with a moistened patch and the trunk of the rabbits was encased with an occlusive wrapping. The Rice Wax had a PII of 0.21 (Leberco Testing Inc., 1991b).

Following the same protocol, Hydrogenated Rice Bran Wax was applied to intact and abraded sites of six white rabbits. The substance had a PII of 0.0 (Leberco Testing Inc., 1993c).

The Consumer Product Testing Co. (1998a) used ten (5M:5F) Hartley strain guinea pigs as a test group in a Magnusson and Kligman guinea pig maximization test of Rice Bran Wax S-100, Lot No. W-90305. An additional ten (5M:5F) Hartley strain guinea pigs were used a control group. For induction, each animal in the test group received three pairs of subcutaneous injections. The three pairs of injection were made in two rows, one row on each side of the midline as follows:

1st pair:	0.1 ml of the TM(Titermax)/water emulsion (1:1), without the test article
2nd pair:	0.1 ml of the test article, at 10% in corn oil
3rd pair:	0.1 ml of the test article/corn oil, in the TM/water emulsion (0.5% test article/4.5% corn oil/47.5% TM/47.5% distilled water).

Seven days later after the injections, an irritating concentration of the test material (25% in petroleum jelly) was topically applied. The suspension (0.5 ml) was spread onto a 2X4 cm piece of filter paper. The filter paper was placed onto the test site and covered with a piece of one and one-quarter inch Blenderm tape. Two weeks after the topical induction application, a challenge application was made. A 5X5 cm area on the flank of each guinea pig, in both the test and control groups, was shaved. The test article, 12.5% in petroleum jelly, which was topically screened as the highest non-irritating concentrations was applied (0.4 ml) to each site with a cotton patch. The animals were wrapped

after the dosing and were set in place for 24 h. Twenty-one hours after the wraps were removed, any remaining test material was removed with an ethanol wipe. Three and twenty-four hours later each site was observed and scored for erythema and edema. The indicies of incidence and severity were calculated for both groups.

Incidence and severity indicies at both 48 and 72 h after applications were 0 in test and control groups. The test material was not a sensitizer in guinea pig under the conditions of this test (Consumer Product Testing Co., 1998a).

The Consumer Product Testing Co. (1998b) also used ten (5M:5F) Hartley strain guinea pigs as a test group in a Magnusson and Kligman guinea pig maximization test of Rice Bran Wax B-10, Lot No. M610117 (hydrogenated rice bran wax). An additional ten (5M:5F) Hartley strain guinea pigs were used a control group. For induction, each animal in the test group received three pairs of subcutaneous injections. The three pairs of injection were made in two rows, one row on each side of the midline as in the previous study. Seven days later after the injections, an irritating concentration of the test material (25% in petroleum jelly) was topically applied. The suspension (0.5 ml) was spread onto a 2X4 cm piece of filter paper. The filter paper was placed onto the test site and covered with a piece of one and one-quarter inch Blenderm tape.

Two weeks after the topical induction application, a challenge application was made. A 5X5 cm area on the flank of each guinea pig, in both the test and control groups, was shaved. The test article, 12.5% in petroleum jelly, which was topically screened as the highest non-irritating concentrations was applied (0.4 ml) to each site with a cotton patch. The animals were wrapped after the dosing and wraps left in place for 24 h. Twenty-one hours after the wraps were removed, any remaining test material was removed with an ethanol wipe. At 48 and 72 hours after dosing, each site was observed and scored for erythema and edema. The indicies of incidence and severity were calculated for both groups.

Incidence and severity indicies at both 48 and 72 h after applications were 0/0.10 (test/control groups). The test material was not a sensitizer in guinea pig under the conditions of this test (Consumer Product Testing Co., 1998b).

Six New Zealand white rabbits each received a single dermal application of 0.5 g of Rice Bran Wax S-100 (Lot #W90305) on two test sites, one abraded and one non-abraded. The test sites were occluded for 24 h and were observed individually for erythema, edema, and other effects 24 and 72 h after application. Mean scores from the 24 and 72 h readings were averaged to determine the primary irritation index. The test article was moistened with saline upon dosing. The primary irritation index was 0.05; the test substance was not a primary dermal irritant (Consumer Product Testing Co., 1998e).

Ocular Irritation

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil

Leberco Testing Inc. (1993d) instilled Rice Bran Oil (0.1 ml) into the conjunctival sac of one eye of each of six albino rabbits. The contralateral eye served as the control. Eyes were graded at 24, 48 and 72 h post-instillation. The substance was considered a primary ocular irritant if $\geq$ four rabbits had a response in the cornea, iris or conjunctiva; the substance was not an irritant if $\leq$ one rabbit had a response, and inconclusive if 2-3 rabbits had a response.

Two rabbits had conjunctiva redness scores of 1 (defined as "some vessels definitely injected"; scale 0-3 with scores $\geq$ 2 considered positive) at the 24 h observation. The condition cleared in one rabbit by 48 h and cleared in the second rabbit by 72 h. The test material was not considered a primary irritant (Leberco Testing Inc., 1993d).

Undiluted Rice Bran Oil was instilled into the conjuctival sac of the eye of six rabbits. Reactions were scored according to the Draize scale (maximum score 110) on days 1, 2, 3, 4, and 7 after instillation. No reactions were noted in the cornea or iris at any observation. One rabbit had a conjunctival score of "2" on days 1 and 2, and another rabbit had a conjunctival score of "2" on days 4 and 7. Rice Bran Oil was considered minimally irritating (CTFA, 1983).

A face lotion containing 8.0% Rice Bran Oil was instilled into the conjunctival sac of three rabbits. No reactions were observed one and two days after instillation (CTFA, 1987a).

Oryza Sativa (Rice) Germ Oil

A mixture of Rice Bran Oil and Rice Germ Oil (0.1 ml) was instilled into the right conjunctival sac of three female albino rabbits. The left eye served as control. Both eyes were rinsed five minutes after instillation. The cornea, iris, and conjunctiva were examined according to the modified Draize Method at 1, 4, and 24 h, and 4 and 7 days after application. Corneal opacity/area of opaque field scores of 1 were observed in the treated eye of all three rabbits throughout the observation period. One rabbit also had erythema/edema scores of 1 at the 1 and 4 h observation; the reaction cleared thereafter. The material was not considered an ocular irritant (Ichimaru Pharcos Co., 1981c).

Six albino rabbits had 0.01 ml of Rice Germ Oil instilled into the conjunctival sac of the test eye. The contralateral eye served as a control. Both eyes were examined and graded at 24, 48, and 72 h post instillation. No ocular lesions or reactions were observed. This material was not considered a primary irritant (Celsis, 1999).

Oryza Sativa (Rice) Wax and Hydrogenated Rice Bran Wax

Rice wax was tested using the above protocol. Three rabbits had conjunctiva redness scores of 1 at the 24 h observation; the redness cleared in all by the 48 h observation. Rice Wax was not considered a primary irritant (Leberco Testing Inc., 1991c).

Hydrogenated Rice Bran Wax was also tested following the same protocol except that additional observations were made at 4 and 7 days post-instillation. Four rabbits had conjunctival redness scores of 1 at the 24 h observation; one of these rabbits also had a conjunctival discharge score of 1. The discharge cleared in two rabbits by 48 h, and in a third rabbit by 72 h. It persisted in the fourth rabbit throughout the observation period, increasing to a score of 2 (considered positive) at the day four reading and returning to a score of 1 at the day seven reading. Hydrogenated Rice Bran Wax was not considered a primary ocular irritant (Leberco Testing Inc., 1993e).

Six New Zealand white rabbits, free from visible ocular defects, each received a single intraocular application of 0.1 ml of Rice Bran Wax S-100 (Lot. L9807091) into one eye. The contralateral eye, remaining untreated, served as a control. The eyes of all animals remained unwashed for 24 h. Observations of corneal opacity, iritis, and conjunctivitis were recorded 24, 48, and 72 h after treatment, and at four and seven days if irritation persisted. The test article was used as a 30% suspension in mineral oil. The average Draize scores at 24, 48, and 72 h were 2.3, 2.0, and 0, respectively. The test article was not an ocular irritant (Consumer Product Testing Co., 1998c).

Six New Zealand white rabbits, free from visible ocular defects, each received a single intraocular application of 0.1 ml of Rice Bran Wax S-100 (Lot. W90305) into one eye. The contralateral eye, remaining untreated, served as a control. The eyes of all animals remained unwashed for 24 h. Observations of corneal opacity, iritis, and conjunctivitis were recorded 24, 48, and 72 h after treatment, and at four and seven days if irritation persisted. The test article was used as a 30% suspension in mineral oil. The average Draize scores at 24, 48, and 72 h and 4 and 7 days were 2.0, 1.3, 0.7, 0.7, and 0 respectively. The test article was not an ocular irritant (Consumer Product Testing Co., 1998d).

Phototoxicity/ Photoprotection

γ-Oryzanol

Ethanol solutions of oryzanol or p-aminobenzoic acid (PABA) were applied to the shaved backs of guinea pigs. One half of each back was then irradiated at a distance of 10 cm with three lamps arranged in parallel (270-320 nm and 320-400 nm). The UV irradiated side was divided into four exposure sections according to minimal erythema dose (MED) levels. At a dose of 3 MED, 100 µg/cm ^{2} oryzanol had the protection activity of 50 µg/cm ^{2} PABA (Ichimaru Pharcos, unknown date).

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil

According to Rukmini and Raghuram (1991), stearic acid comprises 2.9% and tocopherols comprise a very small fraction of Rice Bran Oil.

The CIR Final Report on Oleic Acid, Lauric Acid, Palmitic Acid, Myristic Acid, and Stearic Acid reported a 2.8% formulation of stearic acid in two phototoxicity studies using male guinea pigs to be non-photosensitizing (Andersen, 1987).

Ichimaru Pharcos Co., Ltd. (1997) studied Oryza Oil S-1 (Rice Bran Oil) in a photo-contact allergy

test on 10 female Hartley guinea pigs. The oil was administered undiluted when conducted in the sensitization phase, and was used at a 10% concentration adjusted with Vaseline when conducted in the challenge phase. The Adjuvant-Strip method was used in this study. Each 0.1 ml of an emulsified mixture of distilled water and Freund's Complete Adjuvant of the same amount was injected intracutaneously into the corner of a square (2X4 cm) on the animal's shaved neck. Following the injection, the portion of the neck where the horny layer had been exfoliated by cellophane adhesive tape and 0.1 ml or 0.1 g of the test material had been applied in open condition was irradiated by long wave ultraviolet light (10 J/cm^2) for five days once a day.

Three weeks following the photosensitization the test material (0.02 mg or 0.02g) was applied to an area of 1.5X1.5 cm^2 under non-occlusive conditions and was irradiated again at 10 J/cm^2. Again the sheared skin of the neck was used and a control portion of the skin was covered by aluminum foil while being irradiated. Evaluations were made at 24 and 48 h after irradiation. No signs of erythema or edema were observed at either time interval. The test material was considered negative in this study (Ichimaru Pharcos Co., Ltd., 1997).

The CIR final safety assessment of tocopherol and related ingredients reported that tocopherol acetate was not phototoxic in a study of eleven human subjects (CIR, 1999a).

Oryza Sativa (Rice) Germ Oil

A 5% emulsion of a mixture of Rice Bran Oil and Rice Germ Oil (0.1 ml) was applied to the clipped back of six female Hartley guinea pigs. After 4 hours, half of the test site was irradiated with the minimum erythemogenic dose (MED) provided by three UV 280-320 nm lamps and three UV 320-400 nm lamps placed in parallel at a distance of 10 cm. The other half of the test site was covered with aluminum foil. Sites were evaluated at 24 and 48 h after irradiation. No phototoxic effects were observed (Ichimaru Pharcos Co., 1979b).

Celsis (1999) used a group of ten guinea pigs (male and female) in a study of phototoxicity of Germ Oil-K. An adhesive backed patch of closed cell foam with pre-cut holes was applied to the shaven backs and 0.1 ml of 100, 75, 50, and 25% of Germ Oil-K was placed into four wells. The solutions were left on for 30 min after which the test groups were irradiated for 15 min at 310-400 nm.

One control group of ten animals were prepared as the tested group with doses of 0.1 ml of 100, 75, 50, and 25% of test substance, but not irradiated. Prior to the irradiation exposures, the minimal erythemal dose of light (MED) and minimal erythemal dosage selection were measured. A positive control group using 0.005% 8-Methoxysoralen was also tested. Skin reactions were scored 24 h after irradiation using the Draize scoring table for skin reactions.

Two of ten animals in the 100% test group had a phototoxic response at 24 h. There was no response at 75, 50, or 25% concentrations (in 0.9% sodium chloride) at 24 h. The positive controls produced phototoxic responses in four of the five animals with very slight erythema and no edema at 24 h (Celsis, 1999).

Extracts

Oryza Sativa (Rice) Bran Extract

Safflower Oil was reported as a component of Rice Bran Extract — 97-98.8% solvent: water/propylene glycol, water/butylene glycol, water/glycerin, safflower oil (CTFA, 1999d).

The CIR Final Report of Safflower Oil characterized Safflower Oil was neither a phototoxin nor a photosensitizer based on two clinical studies involving irradiated treatments (Elder, 1985).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil

The three-generation oral-dose study detailed in the Oral Toxicity-Chronic section of this report, also evaluated the reproductive performance of rats fed on 10% Rice Bran Oil. The percentages of conception, birth weight, litter size, weaning weight, and preweaning mortality were comparable with those of rats fed Peanut Oil in both matings in all three generations. Rice Bran Oil was considered safe for human consumption (Rukmini, 1988).

GENOTOXICITY

γ-Oryzanol

γ-Oryzanol was negative in the bacterial DNA repair test (Rec-assay), the Ames test and the rat bone marrow chromosome aberration test. It was also negative in the metabolic cooperation inhibition test using Chinese hamster V79 cells (Tsushimosto et al., 1991).

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil

Rice Bran Oil was tested in the Ames test using *Salmonella typhimurium* strains TA98 and TA100 both with and without metabolic activation. Edible grade oil (200 ml) was shaken continuously with 20 ml dimethyl sulfoxide (DMSO) and then centrifuged. The DMSO layer was separated, stored at 4-10°C and used in the mutagenicity assay. Rice Bran Oil was not mutagenic (Polasa and Rukmini, 1987).

Oryza Sativa (Rice) Wax

Rice Wax was examined for mutagenic activity in a histidine-dependent auxotroph of *Salmonella typhimurium* strain TA 100. The tests were conducted in the absence and presence of hepatic rat microsomes (S9 mixture) and employed a range of concentrations of Rice Wax up to 5000 µg/ml. Positive and negative controls were also used in this study. No increases in revertant colony numbers over concurrent control counts were obtained following exposure to Rice Wax. It was concluded that Rice Wax did not exhibit any mutagenic activity under the conditions of the test (Environmental Technical Laboratory, Ltd., 1998).

CARCINOGENICITY

γ-Oryzanol

γ-Oryzanol was not carcinogenic to either $B6C3F_1$ mice or F344 rats following chronic oral administration (200, 600, or 2000 mg/kg body weight for at least 2 years). Greater incidences of neoplasms were noted in mice and rats of the highest dose groups, but were not statistically significant compared to corresponding controls (Tamagawa et al., 1992a; 1992b).

The published literature recognizes γ-Oryzanol as a naturally occurring antioxidant (Hirose et al., 1991, 1994; Nakamura et al., 1991). Studies that investigated whether it could modify/inhibit the actions of known carcinogens are cited in Table 11.

Bran, Starch, And Powder

Phytic Acid

Because of the use of phytic acid as a natural food additive, Hiasa et al. (1992) studied phytic acid produced from Rice Bran in a two-year drinking water study. Phytic Acid contains 49.1% acid, 14.7% total phosphate, 0.86% inorganic phosphate, < 0.04% chlorides, < 0.072% sulfates, and 0.0003% arsenic compounds soluble in water. Groups of 120 F344 rats (60 each sex) were provided with water containing 1.25% (pH 0.9) or 2.5% (pH 1.15) phytic acid *ad libitum*. Control rats received distilled water. A dose-dependent reduction in mean final body weights was observed in dosed rats. Necropsy was done at the end of the study.

Necrosis and calcification of the renal papillae were noted in treated rats but not in controls. Necrosis was noted in one of 57 males and ten of 55 females of the high-dose group, and in one of 59 males and six of 58 females of the low-dose group. Calcification was noted in three of 57 males and 17 of 55 females of the high-dose group, and in none of 59 males and six of 58 females of the low-dose group.

Papillomas of renal pelvic epithelium developed in three males and four females of the high-dose group and in three females of the low-dose group. The incidence of neoplasms in other organs were comparable to concurrent and historical controls (Hiasa et al., 1992).

CO-CARCINOGENICITY AND ANTI-CARCINOGENICITY

Bran, Starch and Powder

Oryza Sativa (Rice) Bran

F344 rats were fed a 20% bran diet (rice, wheat, corn, or soybean) diet for life. Control rats were fed a no-fiber-added diet. All rats were injected with 1,2-dimethylhydrazine (DMH) at weeks 8 and 10 of age. All surviving rats were killed nine

months after the first DMH dose. Survival was increased in all rats receiving bran diets. The incidence of large bowel neoplasms was 86% in rats fed Rice Bran and 95% in control rats. The difference was not significant (Barnes et al., 1983).

Studies that investigated the anti-carcinogenicity properties of specific components of Rice Bran and Rice Bran Oil are cited in Table 11.

Significant inhibition of carcinogenicity or cytotoxicity of carcinogens was noted with administration of Rice Bran hemicellulose, saccharide, or α-glycan (Takeshita et al., 1992; Takeo et al., 1988). In contrast, γ-oryzanol administered orally to rats did not significantly reduce the incidence of neoplasms (Nakamura et al., 1991; Hirose et al., 1994), and in one study, enhanced the incidence of lung carcinogenesis (Hirose et al., 1991).

CLINICAL ASSESSMENT OF SAFETY

Allergic Reactions

Investigators have noted that rice commonly was regarded as hypoallergenic and was frequently recommended in diets for allergic patients. However, case reports have documented contact urticaria in response to raw rice. A 25 year old female had recurrent attacks of Quincke's edema following ingestion of cereal; intracutaneous testing revealed positive results for some brands of raw rice (van den Hoogenband and van Ketel, 1983).

In another instance, a 17 year old female who threw raw rice at a wedding developed acute erythema of the hands, edema of the eyelids, dyspnea and cough; prick tests, open scratch and handling tests, and radioallergosorbent (RAST) tests were positive for rice (di Lernia et al., 1992). An atopic housewife also developed similar symptoms after handling rice at a wedding as well as during handling of raw rice for cooking. (Lezaun, 1994). Positive responses to other cereal grains were also observed in these rice-positive women.

Ikezawa et al. (1992) reported the creation of a hypoallergenic rice by enzymatic decomposition of the proteins considered to be the major allergens of rice. Forty-four panelists with recalcitrant atopic dermatitis with suspected rice allergy eliminated both rice and wheat-based foods from their diets and ate this new rice for four weeks. The extent of overall skin lesions was expressed by using the atopic dermatitis affected area and severity index (ADASI). A significant decrease in ADASI was observed at observations made during weeks 2 and 4 and at the end of the study. "Moderate" to "remarkable" improvement was observed in 77% of the panelists, and "moderate" to "remarkable" reduction in steroid ointment use was noted. Exacerbation of symptoms was observed in four cases, indicating the new rice still contained some allergens.

Dermal Irritation

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil

In a six-week use study, 30 subjects were instructed to use a moisturizer containing 8.0% Rice Bran Oil for three weeks. Another thirty subjects used a commercially available lotion. After the three week period, all subjects switched to use the "other" lotion for an additional three weeks. Dermatologic exams of the face were conducted at the start and end of the study and at the three-week cross-over. Subjects also answered questionnaires at the end of each three-week use period. The test lotion produced an acceptably low incidence of "skin reactions". However, it produced an unacceptable level of perceived discomfort and/or irritation. Follow-up testing traced the subjective discomfort to silicone fluid contained in the test lotion. The same lotion (containing 8.0% Rice Bran Oil) without the silicone fluid when tested on those with ocular area reactions to the original lotion did not evoke discomfort. The re-formulated lotion was recommended for consumer use (CTFA, 1987b).

Hill Top Research (1989) tested a moisturizer and a body cream each containing 1.04% Rice Bran Oil in a cumulative irritation study. Ten of an original thirteen participants completed the study (two were dropped due to suspected presensitization; one was dropped for reasons unrelated to testing). Each test material (0.2 ml) was applied to a separate area on the back for 23 h of contact.

Table 11. Anti-Carcinogenicity Studies on Components of Rice

Carcinogen/Tumor Cell Administration	Conditions for Rice Administration	Results compared to controls (carcinogen and/or tumor but were fed basal diet)	Reference
	Rice Bran Derived		
groups of 25 male F344 rats; weekly injections of 1,2-dimethylhydrazine at day 35	27 weeks with 2 or 4% rice bran hemicellulose[1] beginning day 0	significant ($p < 0.05$) reduction in colon tumors in 4% rice bran hemicellulose group	Aoe et al., 1993
group of 32 male Wistar rats received N-ethyl-N'-nitro-N-nitrosoguanidine in drinking water for 4 months, followed by 4 months of untreated water	rats received rice bran saccharide (derived from rice bran) at 250 µg/ml in drinking water for 4 months beginning at month 8	gastrointestinal tumors noted in 88% of rats from carcinogen only group and in 46% of rats from carcinogen/rice bran saccharide group ($p < 0.025$). rice bran saccharide also prevented a reduction in immunocompetence, and prolonged survival in rats with cancer	Takeshita et al., 1992; Nakamura, 1992
groups of ten BALB/C mice received subcutaneous inoculations with Meth-A Fibrosarcoma cells (6×10^4 cells/mouse)	α-glucan fractionated from rice bran saccharide days 1-10		Takeo et al., 1988
	oral	10 mg/kg: 21.0% inhibition, ($p < 0.05$) 30 mg/kg: 45.1% inhibition, ($p < 0.01$) 100 mg/kg: 26.2% inhibition, ($p < 0.05$)	
	Intraperitoneal	30 mg/kg: 50.0% inhibition, ($p < 0.01$)	
groups of ten BDF_1 mice received subcutaneous inoculation with Lewis Lung Carcinoma cells (10^5 cells/mouse)	α-glucan fractionated from rice bran saccharide on days 1-10		Takeo et al., 1988
	oral	10 mg/kg: 29.4% inhibition, ($p < 0.05$) 30 mg/kg: 43.8% inhibition, ($p < 0.001$) 100 mg/kg: 27.5% inhibition, ($p < 0.05$)	
	intraperitoneal.	30 mg/kg: 47.9% inhibition, ($p < 0.001$)	
ten BALB/C mice received subcutaneous inoculation with Meth-A	30 mg/kg rice bran saccharide (derived from rice bran) p.o. on days 1-10	48.1% inhibition ($p < 0.01$) :	Takeo et al., 1988
ten BDF_1 mice received subcutaneous inoculation with 3LL	30 mg/kg rice bran saccharide (derived from rice bran) p.o. on days 1-10	46.8% inhibition ($p < 0.001$)	Takeo et al., 1988
	γ-oryzanol		
rats were initiated with intraperitoneal injections of 2,2' dihydroxy-di-n-propyl-nitrosamine; i.g. injections of N-ethyl-N-hydroxynitrosamine; and s.c. injections of 3,2'-dimethyl-4-aminobiphenyl	rats received feed containing 1% γ-oryzanol for 32 weeks	enhancement of lung carcinogenesis noted with microscopic examination	Hirose et al., 1991
male F344 rats initiated with 3,2'-dimethyl-4-aminobiphenyl	rats received feed containing 2% γ-oryzanol for 40 weeks	no significant difference in prostate lesion incidence	Nakamura et al., 1991
Sprague-Dawley rats received intragastric dose of DMBA	rats received feed containing 1% γ-oryzanol for 35 weeks	incidence and multiplicities of mammary tumors comparable to controls; significantly greater survivaf in γ-oryzanol group	Hirose et al., 1994
12-O-tetradecanoylphorbol-13-acetate applied to the outer and inner ears of ICR mice twice weekly for 20 weeks, then 50 ug of 7,12-dimethylbenz-[a]anthracene was applied to the backs of the mice	the methanol extract of rice bran and γ-oryzanol were applied to the ear skin were the TPA was applied	the 50% inhibitory dose of compounds contained in the rice bran oil and γ-oryzanol was 0.2-0.3 mg/ear	Yasukawa et al. 1998

Subjects were instructed to remove the patch, shower, and then report for site evaluation and patch reapplication. Each material was applied to the same site a total of 21 consecutive times. The moisturizer had a total score of 26 and the body cream had a score of 31; the maximum score was 630. Each test material was classified as a mild irritant (Hill Top Research, 1989).

In another test reported by CTFA (1991), twenty females were instructed to apply a body lotion containing 1.04% Rice Bran Oil to the upper chest and neck area twice a day for nine days. One woman developed "significant" follicular irritation. The investigators considered the incidence, "consistent with what has been observed in this assay."

Oryza Sativa (Rice) Wax and Hydrogenated Rice Bran Wax

Nakayama (1976) patch tested 27 subjects with Rice Wax and Hydrogenated Rice Bran Wax. Approximately 73% of the tested Rice Wax was comprised of behenic acid, lignoceric acid, octacosyl alcohol and myricyl alcohol. The composition of the tested Hydrogenated Rice Bran Wax was not reported. Patches containing 3%, 5%, and 10% of both waxes (in a lanolin base) were applied to two sites on the back of each panelist. Patches from one site were removed after 24 h of contact and patches on the other site were removed after 48 h of contact. Sites were evaluated for 1-72 h after patch removal.

Weak positive reactions were noted but were similar to those noted for the lint byssus control, and were not dose-dependent. The investigator considered the results to indicate, "almost no acute primary irritation" (Nakayama, 1976).

Dermal Sensitization and Photosensitization

Oils, Fatty Acids, and Waxes

Oryza Sativa (Rice) Bran Oil

A moisturizer containing 8.0% Rice Bran Oil was tested in a repeat insult patch test (RIPT) using 84 females and 10 males. Nine induction patches were applied to the same site during a three week period. Subjects were instructed to remove the patches after 24 h of exposure. Sites were evaluated prior to application of successive patches. After a three-week non-treatment period, subjects were challenged with a single 24 h patch applied to a previously unexposed area. Reactions were scored 24 and 48 h after patch removal. Twenty-seven subjects had "barely perceptible" or "mild" reactions during induction; in fourteen of these subjects a reaction was noted only at one observation. No reactions were observed at challenge (CTFA, 1987c).

CTFA (1985b) also reported results of testing two formulations each containing 1.04% Rice Bran Oil in RIPTs following the above described protocol. A bath oil was tested as a 10% aqueous dispersion using 87 females and 6 males. Seventeen subjects had instances of "barely perceptible" or "mild" reactions during the induction period. One panelist had a "barely perceptible" reaction at the 24 h challenge evaluation. The bath oil had no allergic sensitization potential (CTFA, 1985a). A body cleanser was tested as a 0.5% aqueous solution on 85 females and 9 males. Ten subjects had instances of "barely perceptible" or "mild" reactions during the induction period. One panelist had a "barely perceptible" reaction at the 24 h and 48 h challenge evaluations.

Hill Top Research (1988) tested a lip balm containing 1.04% Rice Bran Oil in an RIPT using 90 subjects. A total of ten 24 h induction exposures were applied to the same site on the back over a 22 day period. After a two-week non-treatment period subjects were challenged and reactions were scored 48 and 96 h after application. One panelist reacted throughout the induction period and at challenge. The lip balm was considered negative by the authors.

A face/body cream containing 1.04% Rice Bran Oil was tested in a RIPT; 100 subjects completed the protocol. A total of nine 24 h induction patches were applied to the back within a three-week period. Following a two-week non-treatment period subjects were challenged. No reactions were noted during induction or at challenge (AMA Laboratories, 1989).

Ivy Laboratories (1996) tested twenty-five healthy, Caucasian adult volunteers ranging in age from 18 to 57 years completed a photocontact allergenicity test. None of the subjects had a medical or dermatological illness and none were sensitive to sunlight or to topical preparations and/or cosmetics. The test sample used in this study contained 1.04% Rice Bran Oil in a lip balm.

The MED of each subject was determined by exposing one side of the midback to a series of exposures (1 cm diameter in circular areas) in 25% increments from the xenon arc solar simulator. The subject's MED is the time of exposure that produces a minimally visible erythema at 20 to 24 h post-exposure.

The test material (80 mg) was applied to a designated skin site measuring 2X2 cm over the lower back using plastic 1 cc disposable tuberculin syringes. The sites were then covered by squares (2X2 cm) of non-absorbing cotton cloth and the patches fastened to the skin with overlapping strips of occlusive tape. The patches were left in place for 24 h. At the end of the period, the patches were removed and the sites wiped off with dry gauze and then exposed to three MED's from the xenon arc solar simulator.

The sites were then left open for a for a 48 h period and then the patches were reapplied to the same designated test site under an occlusive dressing. Twenty-four hours later, the patches were again removed and the sites re-exposed to another dose of 3 MED's of solar simulated radiation. This sequence was repeated for the same test sites twice weekly for a total of three weeks. Ten to fourteen days following the last induction exposure, the subjects returned to the testing facility for a single challenge exposure.

The test materials were then applied as previously specified (80 mg) in duplicate to new designated skin sites measuring 2X2 cm on the opposite side of the lower back, under an occlusive dressing for a period of approximately 24 h. One set of patches was then removed and any excess material wiped off with dry gauze. Each site was then irradiated with 4 J/cm^2 of UVA. The duplicate set of patches remained unirradiated and served as control unexposed treated sites. All test sites were examined for reactions at 48 h and 72 h following exposure of the sites to UVA radiation.

No reactions were seen during the induction phase, except for some mild erythema, desquamation and tanning which are to be expected following repeated exposures to 3 MED's. No untoward or abnormal reactions of any kind were seen following the challenge in any of the 25 panelists. Under the presently described test conditions, the above test material was not found to possess a photocontact sensitizing potential in human skin (Ivy Laboratories, 1996).

The Consumer Product Testing Co. (1997) tested a children's shampoo and conditioner containing 0.3% w/w Rice Bran Oil in an RIPT using 111 subjects (90 females, 21 males). The shampoo was prepared as a 10% dilution using distilled water. The test materials (~ 2 ml) were applied in semi-occlusive patches to the upper back. Subjects were instructed to remove patches after 24 h. Patching was done three times per week for a total of ten applications. Sites were evaluated prior to application of each subsequent patch. Following a two-week non-treatment period, subjects were challenged at both the induction patch application site and at an unexposed site on the volar forearm. Sites were evaluated at 24 and 48 h after application.

Nine subjects (seven females, two males) dropped out of the study for reasons unrelated to the test material. Mild erythema in response to the conditioner was observed in one subjects at the fourth and fifth induction observation. No reactions were observed at challenge. The shampoo and conditioner, "did not indicate a potential for dermal irritation and/or sensitization" (Consumer Product Testing Co., 1997).

Ivy Laboratories (2000) conducted a photocontact allergenicity potential assay in twenty-five healthy Caucasian subjects (17 males and 9 females) ranging in age from 18 to 49 years. All subjects had skin types ranging from I to III (burns easily; never tans to burns moderately, tans gradually). The test sample contained 1.5% Rice Bran Oil in a lotion. None of the subjects had a medical or dermatological illness and none were sensitive to sunlight or to topical preparations and/or cosmetics.

The test patches were applied to the lower back of each subject. In the pre-testing phase, the MED (minimal erythema dose) of each subject was determined by exposing one side of the midback to a series of exposures (1 cm diameter circular areas) in 25% increments from a xenon arc solar simulator. Approximately 40 mg of the test material was applied to 2X2 cm square skin sites over the lower back and covered with non-woven cotton cloth. The patches were fastened to the skin with occlusive tape and left in place for 24 h. At the end of this period, the patches were removed and the sites were wiped off with dry gauze and exposed to three MED's from the xenon arc solar simulator. The sites were then left open for a 48 h period, after which the patches were reapplied to the same designated

test site under an occlusive dressing. Twenty-four hours later, the patches were removed and the sites re-exposed to 3 MED's of solar simulated radiation. This sequence was repeated to the same test sites twice weekly for a total of six weeks (total of 6 exposures).

Twelve days following the last induction dose, the subjects returned for a single challenge exposure. The test material was applied as previously specified (40 mg) in duplicate to new designated skin sites measuring 2X3 cm on the opposite side of the lower back, under occlusive dressings for a period of approximately 24 h. One set of patches was then removed and any excess test material was wiped off with gauze. The sites were then exposed to 4 J/cm^2 of UVA light (spectrum between 320 and 420 nm). The duplicate patches remained unirradiated and served as controls. All test sites were examined for reactions at 48 and 72 h following exposure of the site to the UVA radiation.

No side-effects or unexpected reactions of any kind were observed. No reactions suggestive of a photocontact allergy was seen in any panelist at either 48 or 72 post exposure. Under the present conditions described, the test materials in the lotion did not possess a detectable photocontact-sensitizing potential in human skin (Ivy Laboratories, 2000).

Bran, Starch, and Powder

Oryza Sativa (Rice) Bran

Pigatto et al. (1997) conducted a double-blind, randomized patch study to investigate whether colloidal grain suspensions induced allergic contact dermatitis in atopic children. Initially, a 15 minute open-patch of a colloidal rice flour solution was applied to the back of 65 children aged 6 months to 2 years (43 were atopic and 22 were normal). As no urticarial reponse was observed, occlusive patches containing 0.007% and 0.7% colloidal rice flour were applied. If no positive response was observed at 24 h, the contralateral patch remained in place for another 24 h. Sites were evaluated at the time of patch removal and also at 72 and 96 h.

One atopic child had a mild irritant reponse to the 0.007% rice solution at 48 h, but no allergic reactions were observed in any of the children. RAST tests were done on 55 children. Eight had a positive reponse to one of the test substances (details not given); these eight were atopics. The investigators considered that topical colloidal grains did not induce sensitization (Pigatto et al., 1997).

Therapeutic Use

Fujiwaki and Furusho (1992) investigated the therapeutic value of Rice Bran broth-bathing in treating atopic dermatitis. Broth was prepared by boiling rice bran with water and then cooling and filtering the mixture. Seventeen subjects with mild to severe atopic dermatitis were instructed to mix 1 L of the broth with bath water, once a day followed by a shower with fresh water. The therapy continued for 2-5 months. Subjects were evaluated before starting therapy, 2 weeks and 1 month after therapy initiation, and monthly thereafter. In five patients serum IgE concentrations and eosinophil counts (from peripheral blood) were measured prior to and 2-3 months after therapy initiation.

One subject developed redness and itching just after bathing and discontinued therapy. None of the remaining 16 subjects had adverse effects. A significant decrease in the dermatitis score was noted after two weeks of therapy and no subject had a recurrence of his/her initial disease. One subject's dermatitis improved such that steroid ointment-treatment was no longer needed. In another two subjects the dosage and grade of steroid treatment was reduced, and another three had a reduction in either dosage or grade of ointment. Of the 16 subjects who completed the protocol, therapy was considered to be excellent in four, good in seven, slightly effective in four, and ineffective in one. A non-significant decrease in IgE concentrations and a significant decrease ($p < 0.05$) in eosinophil counts were observed with therapy (Fujiwaki and Furusho, 1992).

SUMMARY

Oryza Sativa (Rice) Bran Oil, Oryza Sativa (Rice) Germ Oil, Rice Bran Acid, Oryza Sativa (Rice) Wax, Hydrogenated Rice Bran Wax, Oryza Sativa (Rice) Extract, Oryza Sativa (Rice) Bran Extract, Oryza Sativa (Rice) Germ Powder, Oryza Sativa (Rice) Starch, Oryza Sativa (Rice) Bran, Hydrolyzed Rice Bran Protein, and Hydrolyzed Rice Protein are derived from rice *Oryza sativa*.

Animal and clinical studies have reported that consumption of Rice Bran Oil or Rice Bran had protective effects on blood parameters. Sunscreen patents have described using Rice

Bran Oil and Rice Wax. A Rice Bran Oil and Rice Germ Oil mixture had a UV absorption maximum at 315 nm.

Oils, Fatty Acids, and Waxes

Rice Bran Oil functions in cosmetics as a conditioning agent and solvent and was used in 34 formulations in 2001. Rice Germ Oil functions as a skin-conditioning agent and was reported in body and hand creams, lotions, powders, and sprays. Rice Bran Acid functions as a surfactant -cleansing agent and was not reported in use. The two Bran Waxes function as binders, conditioning agents, and viscosity increasing agents and were used in 17 formulations.

Rice Bran Oil had an oral LD_{50} of > 5 g/kg in white rats and Rice Wax had an oral LD_{50} of > 24 g/kg in male mice. A three-generation oral dosing study reported no toxic or teratologic effects in albino rats fed 10% Rice Bran Oil compared to a control group fed Peanut Oil. In primary dermal irritation studies, undiluted Rice Bran Oil had a PII of 0.00 and 0.88, Rice Wax had a PII of 0.21, and Hydrogenated Rice Bran Wax had a PII of 0.0 (scores > 5.0 were considered irritants). Rice Germ Oil did not produce dermal irritation and Rice Bran Oil was not a sensitizer. Rice Bran Oil, Rice Germ Oil, Rice Wax, and Hydrogenated Rice Bran Wax were negative in ocular toxicity assays. A mixture of Rice Bran Oil and Rice Germ Oil was not phototoxic in a dermal exposure assay.

Rice Bran Oil was negative in an Ames assay, and a component, γ-oryzanol, was negative in bacterial and mammalian mutagenicity assays.

Formulations containing 1.04% or 8.0% Rice Bran were at most mildly irritating in clinical studies. Rice Bran Oil was negative in six RIPTs (maximum concentration tested was 8.0%). Rice Wax and Hydrogenated Rice Bran Wax were patch tested and produced "almost no acute primary irritation" in 27 subjects.

Extracts

Rice Bran Extract functions as a biological additive and was used in five formulations, Rice Extract functions as a biological additive and was in four formulations.

Rice Extract reduced the cytotoxicity of sodium chloride in male rats.

Bran, Starch and Powder

Rice Bran functions as an abrasive and bulking agent and was used in one formulation, Rice Starch functions as a skin conditioning agent - occlusive and was used in 64 formulations; Rice Germ Powder functions as an abrasive and was reported in one use for exfoliating purposes.

Oral-dose carcinogenicity studies done on components of Rice Bran, phytic acid and γ-oryzanol were negative. Rice Bran did not have an anti-carcinogenic effect on DMH-induced large bowel tumors. In co-carcinogenicity studies done using known tumor initiators and Rice Bran Oil and Rice Bran-derived hemicellulose and saccharide, significant tumor inhibition was observed; γ-oryzanol did not inhibit the development of neoplasms.

A decrease in cutaneous lesions in atopic dermatitis patients was reported following bathing with a Rice Bran preparation.

Proteins

Hydrolyzed Bran Protein and Hydrolyzed Rice Protein function as conditioning agents (hair or skin). No uses were reported. Isolated cases of allergy to raw rice have been reported.

DISCUSSION

In its original final report on the safety assessment of these ingredients, the CIR Expert Panel had concluded that Oryza Sativa (Rice) Germ Powder and Oryza Sativa (Rice) Starch are safe as used in cosmetic products. Data were available supporting the absence of any significant animal or human toxicity of these materials. This conclusion was supported by the available data on Wheat Starch previously considered by the Panel.

The CIR Expert Panel considered new data on the Oils, Fatty Acids, and Waxes along with existing data within the document. These data show the fatty acid composition of these ingredients to include fatty acids previously determined safe by the CIR Expert Panel. In addition animal and human toxicity data of the rice-derived Oils, Fatty Acids, and Waxes did not suggest any toxicity. Based on all the available information, the CIR Expert Panel concluded that the following ingredients Oryza Sativa (Rice) Bran Oil, Oryza Sativa (Rice) Germ Oil, Rice Bran

Acid, Oryza Sativa (Rice) Wax, and Hydrogenated Rice Bran Wax are safe as used in cosmetic formulations.

The available data, however, on the starches, Oils, Fatty Acids, and Waxes could not be extended to extracts or the proteins derived from rice. Section 1, paragraph (p) of the CIR Procedures states that "A lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the Procedures, the Expert Panel informed the public of its decision that the data on Oryza Sativa (Rice) Bran Extract, Oryza Sativa (Rice) Extract, Oryza Sativa (Rice) Germ Powder, Oryza Sativa (Rice) Starch, Oryza Sativa (Rice) Bran, Hydrolyzed Rice Bran Protein, Hydrolyzed Rice Protein were not sufficient for determination whether the ingredients, under relevant conditions of use, were either safe or unsafe.

Data needed to make a safety assessment are:

For Oryza Sativa (Rice) Bran Extract and Oryza Sativa (Rice) Extract:

1. UV absorption; if there is significant absorption, then a dermal phototoxicity/photosensitization study will be needed
2. dermal irritation and sensitization at concentration of use
3. ocular irritation, if available

Data regarding Rice Extract would apply to both ingredients, while data regarding Rice Bran Extract would only be relevant to that ingredient.

For Oryza Sativa (Rice) Bran:

1. concentration of use
2. contaminants (pending results, additional studies may be needed)
3. UV absorption; if there is significant absorption, then a dermal phototoxicity/photosensitization study will be needed
4. dermal irritation and sensitization at concentration of use
5. ocular irritation, if available

Hydrolyzed Rice Bran Protein, and Hydrolyzed Rice Protein the data needed are:

1. method of extraction
2. UV adsorption, if there is significant absorption, then a dermal phototoxicity/photosensitization study will be needed
3. dermal irritation and sensitization at concentration of use
4. ocular irritation, if available

Data regarding Hydrolyzed Rice Protein would apply to both ingredients, while data regarding Hydrolyzed Rice Bran Protein would only be relevant to that ingredient.

Note: These rice derived ingredients as used in products should not contain significant levels of pesticide residues or heavy metals.

The Panel is concerned about contaminants such as pesticides and heavy metals. Limitations for such contaminants can be found on page 13 of this document.

CONCLUSION

The CIR Panel concludes that the available data are sufficient to support the safety of Oryza Sativa (Rice) Germ Powder, Oryza Sativa (Rice) Starch, Oryza Sativa (Rice) Bran Oil, Oryza Sativa (Rice) Germ Oil, Rice Bran Acid, Oryza Sativa (Rice) Wax, and Hydrogenated Rice Bran Wax in cosmetic formulations and the available data are insufficient to support the safety of Oryza Sativa (Rice) Bran Extract, Oryza Sativa (Rice) Extract, Oryza Sativa (Rice) Bran, Hydrolyzed Rice Bran Protein, and Hydrolyzed Rice Protein for use in cosmetic products.

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