APPLICATION FOR THE APPROVAL OF MAGNOLIA BARK SUPERCRITICAL CARBON DIOXIDE EXTRACT (MBSE) FROM *MAGNOLIA OFFICINALIS*

Under

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 Concerning Novel Foods and Novel Food Ingredients

September 2, 2009

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ADMINISTRATIVE DATA

Name and Address of Applicants/Manufacturers

The application is submitted by:

The William Wrigley Jr. Company
410 N. Michigan Avenue
Chicago, IL
60611

Name and Address of Person(s) Responsible for Dossier

GENERAL INTRODUCTION

The William Wrigley Jr. Company proposes to market confectionary products containing Magnolia Bark Supercritical Carbon Dioxide Extract (MBSE), derived from the bark of the plant *Magnoliae officinalis,* subspecies *biloba*, (*Magnoliae officinalis)*. Compositionally, the powered extract is comprised of two phenolic compounds, *magnolol* and *honokiol*. Approval is sought under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients (hereafter referred to as EC 258/97), and accordingly, this submission has been prepared pursuant to the Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients (hereafter referred to as the Commission Recommendation of 1997) (European Parliament and the Council of the European Union, 1997).

Article 1(2.) of EC 258/97 states that the regulation "…shall apply to the placing on the market within the Community of foods and food ingredients which have not hitherto been used for human consumption to a significant degree within the Community and which fall under the following categories…(e) foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating and breeding practices and which have a history of safe food use;". MBSE is thus considered a novel food/food ingredient due to the isolation of the extract from the *Magnoliae officinalis* plant (European Parliament and the Council of the European Union, 1997).

Section 4 of the Commission Recommendation of 1997 outlines recommendations made by the Scientific Committee on Food (SCF) pertaining to the "Scientific Classification of Novel Foods for the Assessment of Wholesomeness", which facilitates the safety and nutritional evaluation of a given novel food/food ingredient.

Of the 6 classes identified, MBSE would be classified as Class 2 "Complex Novel Food from non-GM source", since the production of MBSE is developed by conventional techniques, and with no use of genetic modification.

Since the proposed use of MBSE in chewing gum and compressed mints, as proposed by the William Wrigley Jr. Company, has not been introduced to the community, MBSE can be further allocated under Sub-Class 2.2: "the source of the novel food has no history of food use in the Community". The essential information requirements corresponding with this classification are outlined in a detailed list below, and are expanded upon in separate sections throughout the document, forming the basis of the application (Recommendation 97/618/EC - Commission of the European Communities, 1997).

I Specification of the Novel Food

II Effect of the Production Process Applied to the Novel Food

III History of the Organism Used as the Source of the Novel Food

IV-VIII Not Applicable

IX Anticipated Intake/Extent of Use of the Novel Food

X Information from Previous Human Exposure to the Novel Food or its Source[1]

XI Nutritional Information on the Novel Food

XII Microbiological Information on the Novel Food

XIII Toxicological Information on the Novel Food

For each category (I through XIII), structured schemes have been developed by the SCF, which consist of a decision-tree-like set of questions designed to elicit sufficient data for a comprehensive safety and nutritional evaluation of the novel food. As outlined below in Sections I through XIII, the required questions are identified and subsequently addressed with the appropriate data.

As detailed herein, the safety of MBSE is supported by the purity of MBSE (chemical purity >95%), the historical consumption of plant lignans in the diet, minimal exposure under the conditions of intended use, safety data provided on the final MBSE product, and safety data from additional published and unpublished toxicological and clinical data.

[1] Although this category is not required for Class 2.2 Novel Foods and food ingredients, it has been included in this application since exposure to crude extracts of Magnolia bark has a history of use in traditional Asian remedies this category was considered relevant.

I SPECIFICATIONS OF MAGNOLIA BARK EXTRACT (MBSE)

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to the specifications of the novel food:

- "Is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?"
- "Is the information representative of the novel food when produced on a commercial scale?"
- "Is there an appropriate specification (including species, taxon *etc.* for living organisms) to ensure that the novel food marketed is the same as that evaluated?"

These questions have been addressed collectively in Sections I.a through I.f.

I.a Common Name or Usual Name

- Magnolia Bark Supercritical Carbon Dioxide Extract (MBSE)
- MBSE is comprised (≥94%) of the following two compounds:
    1. Magnolol
    2. Honokiol

I.b Chemical Name and Chemical Abstract Service (CAS) Number

The chemical names for the two major components of MBSE are:

- 5,5'-diallyl-2,2'dihydroxybiphenyl (Magnolol)
- 5,3'-diallyl-2,4'-dihydroxybiphenyl (Honokiol)

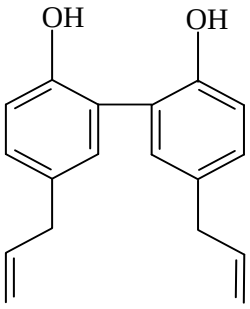
The two major components of MBSE have the following CAS numbers:

- 528-43-8 (Magnolol)
- 35354-74-6 (Honokiol)

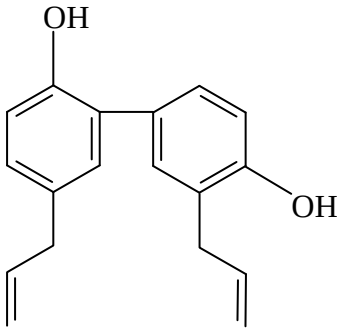
I.c Empirical Formula

MBSE is composed of magnolol and honokiol and both constituents have the same empirical formula of $C_{18}H_{20}O_5$ with a molecular weight of 266.34 daltons.

I.d Structural Formula



Magnolol



Honokiol

I.e Compositional Analyses and Conformance to Product Specifications

MBSE is obtained from *Magnoliae officinalis* bark using a supercritical carbon dioxide (CO_2) extraction method. The supercritical CO_2 extract is dissolved in medical-grade ethanol and re-crystallized yielding a light brownish powder. The product specifications for MBSE are presented in Table I.e-1.

Table I.e-1 Specifications for Magnolia Bark Supercritical Carbon Dioxide Extract

Specification Parameter	Specification	Reference/Test Methodology Performance of Test
Appearance	Light Brownish Powder	-
Magnolol	92.5% min	Internal Validated Methods
Honokiol	0.5% min	Internal Validated Methods
Magnolol + Honokiol	94% min	-
Total Eudesmol	2% max	Internal Validated Methods
Moisture	0.5% max	FCC V, Pg 851
Impurities		
Arsenic (ppm)	0.5 max	FCC V, Pg 861
Lead (ppm)	0.5 max	FCC V, Pg 861
Total Heavy Metals (ppm)	10 max	FCC V, Pg 861
Methyl Eugenol (ppm)	50 Max	-
Tubocurarine (ppm)	2 Max	Internal Validated Methods
Total Alkaloid (ppm)	100 Max	Internal Validated Methods

Table I.e-1 Specifications for Magnolia Bark Supercritical Carbon Dioxide Extract		
Specification Parameter	**Specification**	**Reference/Test Methodology Performance of Test**
Microbial		
Aerobic Count	1000 cfu/g	Most Current ed of FDA-BAM or equivalent
Pathogens	None detected including but not limited to *coliform* bacteria, *salmonella*, *staphylococcus aureus*	Most Current ed of FDA-BAM or equivalent
Yeast and Mould	100 cfu/g	Most Current ed of FDA-BAM or equivalent

*Certificates of analysis and analytical methods for specifications can be found in Appendix A

As shown in Table I.e-2, analysis of 6 lots of MBSE indicate that the manufacturing process produces a consistent product in terms of its chemical composition. Supporting documentation including methods of analysis for magnolol, honokiol, eudesmol, total alkaloids, and tubocurarine is provided in Appendix A.

Table I.e-2 Chemical Analysis of Magnolia Bark Supercritical Carbon Dioxide Extract Manufacturing Lots

Specification Parameter	**Manufacturing Lots**					
	040601	**040602**	**040603**	**050901**	**050902**	**050903**
Appearance	Complies	Complies	Complies	Complies	Complies	Complies
Magnolol (%)	95.7	99.7	96.5	93.4	93.8	93.9
Honokiol (%)	2.3	0.7	1.1	2.0	2.0	2.0
Magnolol + Honokiol	98.0	100.4	97.6	95.4	95.8	95.9
α-, β-, γ-Eudesmol	0.63	0.23	0.58	0.34	0.29	0.63
Moisture (%)	N/A	N/A	N/A	< 0.5%	< 0.5%	< 0.5%
Impurities						
Arsenic (ppm)	< D.L[a]	< D.L[a]	< D.L[a]	< D.L[a]	< D.L[a]	< D.L[a]
Lead (ppm)	< D.L[b]	< D.L[b]	< D.L[b]	< D.L[b]	< D.L[b]	< D.L[b]
Total Heavy Metals (ppm)	< 10	< 10	< 10	< 5	< 5	< 5
Methyl Eugenol (ppm)	< 50	< 50	< 50	6.8	7.5	6.5
Tubocurarine (ppm)	N/A	N/A	N/A	< 2	≤ 2	≤ 2
Total Alkaloid (ppm)	34	< 50	< 50	≤ 5.5	≤ 5.5	≤ 5.5

D.L[a] = Detection limit of 0.5 ppm; D.L[b] = Detection limit of 0.1 ppm ; N/A = Not available/analyzed

I.f Microbiological Analysis of MBSE

A summary of the microbiological product analysis for several batches of MBSE indicating the product is free of microbial contamination is presented in Table I.f-1. Supporting documentation for batch analysis and information pertaining to the methodology are provided in Appendix A.

Table I.f-1 Summary of the Microbiological Product Analysis for Five Lots of MBSE				
Specification Parameter (Methodology)	**Manufacturing Lots**			
	Lot No. 040601	**Lot No. 050901**	**Lot No. 050902**	**Lot No. 050903**
Aerobic Plate Count	<D.L*	<D.L	<D.L	<D.L
Pathogenic Bacteria				
Clostridium (cfu/g) AOAC 975.30	<D.L	<D.L	<D.L	<D.L
Coliform Bacteria (cfu/g) AOAC 991.14	<D.L	<D.L	<D.L	<D.L
E. Coli (cfu/g) AOAC 991.14	<D.L	<D.L	<D.L	<D.L
Salmonella (N/25g) AOAC 993.08	Negative	Negative	Negative	Negative
Staph Bacteria (cfu/g) AOAC 975.55	<D.L	<D.L	<D.L	<D.L
Mould and Yeast				
Mould (cfu/g) FDA-BAM Chapter 18	<D.L	<D.L	<D.L	<D.L
Yeast (cfu/g) FDA-BAM Chapter 18	<D.L	<D.L	<D.L	<D.L

* Detection limit for the above assays was <10 cfu/g

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO MBSE

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

- "Does the novel food undergo a production process?"
- "Is there a history of use of the production process for the food?" If no, "does the process result in a significant change in the composition or structure of the novel food compared to its traditional counterpart?"
- "Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from use of the process?"
- "Are the means identified for controlling the process to ensure that the novel food complies with its specification?"
- "Has the process the potential to alter the levels in the novel food of substances with an adverse effect on public health?"
- "After processing is the novel food likely to contain microorganisms of adverse public health significance?"

These questions have been addressed collectively in Sections II.a through II.c.

II.a Raw Materials Used in the Manufacturing Process

II.a.1 *Magnoliae officinalis*

Magnolia Bark is obtained from the *Magnoliae officinalis* (Subsp. biloba) plant, and the magnolia bark used in the manufacture of MBSE is typically harvested (peeled off the magnolia tree) during the months of April through June. After harvesting, the barks are then cleaned of any dirt or impurities, and dried to produce single or double rolls 30 to 25 cm long and 0.2 to 0.7 cm thick. The dried bark is gray-brown in colour with oval lenticles in a longitudinal striation. The sub-surface, found under the coarse outer layer of bark is yellowish-brown, while the inner surface of the bark is purple-brown or dark purple-brown in colour with delicate longitudinal striations and a smooth surface. The phenolic compounds are contained within the smooth inner layer, which becomes oily when scratched. The bark has a fragrant odour, a pungent taste, and is slightly bitter.

II.a.2 Carbon Dioxide

The carbon dioxide used in the supercritical extraction process is medical grade.

II.a.3 Ethanol

The ethanol used during the recrystallization process is medical grade.

II.b Manufacturing Process

MBSE is obtained from *Magnolia officinalis* bark using a supercritical carbon dioxide extraction method, a widely utilized food manufacturing process. The raw material is cleaned of dirt and debris, and then washed with water. The washed material is dried at 50°C using a hot air circulation drying oven to reduce the moisture content of the bark to less than 9%. The drying step is followed by slicing of the dried product with a reciprocating slicer, and the sliced product then enters a crushing machine. The crushed raw material is placed in a 1,000 L type supercritical carbon dioxide extractor. Medical-grade carbon dioxide gas is pumped into the extractor at a rate of 1,200 to 1,400 L/h over a 3.5-hour period. The extractor temperature is maintained at a temperature and pressure of 35 to 40°C and 25 to 30 MPa, respectively. The supercritical CO_2 extract is then dissolved in medical-grade ethanol and re-crystallized resulting in a product with a total magnolol content of greater than 92.5 % [see specs below]. In addition to providing a product that is highly pure, the supercritical carbon dioxide extraction procedure also ensures that the water soluble curarine alkaloids (magnocurarine) are kept to a minimum in the final product. MBSE is manufactured using current Good Manufacturing Practice, and in accordance with the requirements of Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs, a Hazard Analysis and Critical Control Point (HACCP) program has been implemented for the manufacture of MBSE.

II.c Stability of MBSE

Stability analysis of MBSE in gum and mints was performed under accelerated conditions over a period of 12 weeks. For compressed mints, 0.2% MBSE was incorporated into the product at Wrigley's lab and based on a pellet weight of 0.7 g corresponded to 1.4 mg of MBSE per mint. MBSE was incorporated into gum at a concentration of 0.067% and based on an individual gum weight of 1.5 g corresponded to 1 mg of MBSE per stick of gum. As shown in figure II.c-1, minimal MBSE loss was observed at 12 weeks (94.8±3.4%) relative to time = 0 levels (100% ± 3.4%). MBSE was also observed to be stable in mints, and over a 12 week period, baseline levels (100% ±0.9%) were virtually unchanged (98.9±0.09%) when stored at elevated temperatures and humidity (Figure II.c-2). The high-performance liquid chromatography (HPLC) method used in the analysis was a modified version of a method reported by Tsai and Chen (1992). The assay showed good linearity in the concentration range between 4.05 to 162 µg/mL (R^2=0.99998). The analysis of MBSE from compressed mints and gum displayed a standard deviation of 1.26 and 2.32% respectively.

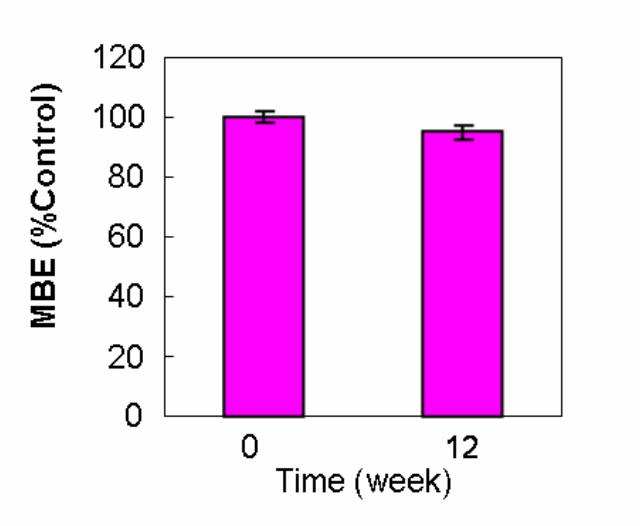


Figure II.c-1 Stability of MBSE in Gum at Week 12 Stored at 85°F and 85% RH.
In gum, minimal MBSE loss was observed at 12 weeks (94.8±3.4%) relative to time = 0 levels (100% ± 3.4%).

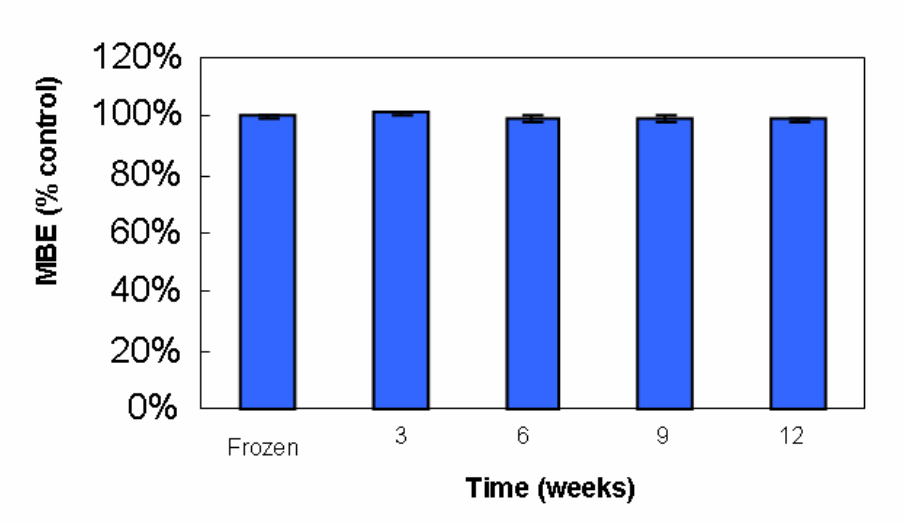


Figure II.c-2 Stability of MBSE in Compressed Mints over 12 Weeks Stored at 95°F.
MBSE was observed to be stable in mints, and over a 12 week period baseline levels (100% ±0.9%) were virtually unchanged (98.9±0.09%) when stored at elevated temperatures and humidity.

III HISTORY OF THE SOURCE ORGANISM OF MBSE – *MAGNOLIAE OFFICINALIS* SUBSP. BILOBA

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the history of the source organism:

- "Is the novel food obtained from a biological source, *i.e.*, a plant, animal or microorganism?"
- "Has the organism used as the source of the novel food been derived using GM?"
- "Is the source organism characterized?"
- "Is there information to show that the source organism and/or foods obtained from it are not detrimental to human health?"

These questions have been addressed collectively in Section III.a and III.b.

III.a Taxonomic Classification of *Magnoliae officinalis*

Magnolia Bark is extracted from *Magnoliae officinalis* subsp. biloba*,* and is not a genetically modified organism. The current taxonomic description of the plant is summarized below:

Kingdom: Viridiplantae
Phylum: *Streotophyta*
Class: *Eukaryota*
Order: *Magnoliales*
Family: *Magnoliaceae*
Genus: *Magnolia*
Species: *Magnolia officinalis*
Subspecies: Biloba

Magnolia bark is the dried stem, root, or branch bark of *Magnoliae officinalis* subsp. biloba of the Family *Magnoliaceae*. Traditionally, magnolia bark also is derived, though less commonly, from *Magnoliae obovata* Thunb (Chang and But, 1986); however, this species is not used in the production of MBSE. Magnolia bark is known as Houpo or Koboku in China and Japan, respectively, and further classified in Japan as Kara-koboku (*M. officinalis*) or Wa-koboku (*M. obovata*) depending on the species of magnolia from which the extract is obtained (Kuribara *et al.*, 2000). Characteristically, magnoliaceous plants produce large amounts of isoquinolines, lignans, neolignans, alkaloids, mono and sesquiterpenes (Ito *et al.*, 1982; Tachikawa *et al.*, 2000).

Chemical investigations of the cortex of *M. officinalis* and *M. obovata* led to the isolation of several major phenolic compounds, including the neolignan derivatives magnolol (5,5'-diallyl-2,2'-dihydroxybiphenyl) and honokiol (5,3'-diallyl-2,4'-dihydroxybiphenyl (Figure 1-1), which are considered the two principle phenolic compounds in the bark (Fujita *et al.*, 1972; Zhao *et al.*, 1991; Hsieh *et al.*, 1998; Bang *et al.*, 2000).

Magnolia bark extracts also contain volatile oils, the major constituents of which are the sesquiterpenoid alcohols, α- and β- and γ-eudesmol (Arora *et al.*, 1967; Tachikawa *et al.*, 2000; Matsuda *et al.*, 2001). Specific components in the bark and the proportion of those constituents differ significantly depending on where the bark is cultivated and the time of harvesting (Tachikawa *et al.*, 2000). Commercially available magnolia bark extracts appear to be marketed based on their high phenolic content, and are therefore predominantly comprised of magnolol and honokiol, with levels of these two constituents ranging from 40 to 90%. Evaluations of the quality of commercial Houpo samples from different regions, or following different harvesting and processing procedures, are also based on a quantitative determination of the level of magnolol and honokiol in the bark (Zhao *et al.*, 1991).

III.b Other Dietary Exposures to *Magnoliae officinalis*

Herbal preparations containing Magnolia bark, such as Banxia Houpo Tang, Saiboku-To, Hange-Koboku-To, Hsiao-Cheng-Chi-Tang, and Wuu-Ji-San have been used for centuries as part of traditional Asian remedies (Kampo medicines) (Hattori *et al.*, 1986; Tsai *et al.*, 1995; Ogata *et al.*, 1997; Sarker, 1997; Hsieh *et al.*, 1998; Maruyama *et al.*, 1998). Herbal preparations containing Magnolia bark are typically used at intakes ranging from 3 to 10 g per person in decoction (Chang and But, 1986). Various Magnolia bark derived extract also can be found in the marketplace as ingredients in dietary supplements, typical recommended use levels for these products are between 200 to 800 mg/person/day. Thus, consumption of crude magnolia bark preparations in the diet is limited users of traditional Asian medicine, dietary supplement users, and significant exposure to the bark in the European diet is not expected.

IX INTAKE/EXTENT OF USE OF MAGNOLIA BARK EXTRACT

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the intake/extent of use of the novel food:

- “Is there information on the anticipated uses of the novel food based on its properties?”
- “Is there information to show anticipated intakes for groups predicted to be at risk?”
- “Will introduction of the novel food be restricted geographically?”
- “Will the novel food replace other foods in the diet?”

These questions have been addressed collectively in Sections IX.a through IX.b.

IX.a Conditions of Intended Food Use

Wrigley's MBSE is proposed for use in gum and mints, at a maximum use level of 0.2%. Products containing MBSE will be marketed for their breath freshening capacity, and therefore will be added to select mint and gum products. It should be noted that the intake estimations conducted in section IX.c were conducted using select food codes. These food codes were considered representative of products used by consumers of breath freshening products, rather than products selected by "candy consumers". Thus, large sugar containing products like "Hubba Bubba" and "Everton" mints were not used in the gum and mint intake estimations respectively since Wrigley does not intend to add MBSE to these types of confectionary products, nor were these types of products considered representative of mint and gum products used by consumers of breath freshening products.

Based on a maximum gum and mint size of 1.5 g, each gum and mint serving would contain a maximum of 3 mg of MBSE. Gum and Mint products containing MBSE will not be geographically restricted, and are intended to be marketed throughout the E.U. It should be emphasized that the proposed use of MBSE in gum and mints is limited to its breath freshening capacity, and these products will not be marketed with claims related to medicinal benefits, nor are medicinal effects expected under the proposed food uses[2]. A comparison of the use of crude Magnolia bark extracts in traditional Asian remedies and comparison to Wrigley's product was provided to the U.K. Medicines and Healthcare Products Regulatory Agency (MRHA) to provide assurance that the proposed use of MBSE would not have medicinal effects, and therefore qualifies as a food ingredient. A copy of the report and response from the Agency is included in Appendix B.

The individual proposed food-uses and use-levels for MBSE employed in the current intake analysis are summarized in Table IX.a-1.

Table IX.a-1 Summary of the Individual Proposed Food-Uses and Use-Levels for MBSE in the U.K.

Proposed Food-Use	Serving Size*	MBSE (mg/serving)	Use-Level (%)
Mints#	1.5 g	3	0.2
Chewing Gum†	1.4 g	2.8	0.2

Small sugar-free type mints
* Actual product sizes will vary, maximum serving sizes are shown.
† MBSE will be incorporated into the outer candy coating of the gum

[2] The following patent applications provide additional support for the non-therapeutic application of MBSE US 20040081713 - Breath freshening and oral cleansing product with magnolia bark extract ; US 20060013779 - Breath freshening and oral cleansing product with magnolia bark extract in combination with surface active agents; US 20060275222 - Breath freshening and oral cleansing products with synergistic combinations of magnolia bark extract and essential oils; US 20080107610 - Breath freshening and oral cleansing product with Magnolia Bark Extract

IX.b Food Labelling Instructions

MBSE shall be displayed on the labelling of the food product as such or in the list of ingredients of foodstuffs containing it, in accordance with the requirements of Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs (as amended)

IX.c Estimated Consumption of MBSE from Proposed Food Uses

The Ministry of Agriculture, Fisheries, and Food (MAFF) and the Department of Health were responsible for the joint commission of the National Diet and Nutrition Survey (NDNS) program in 1992. The responsibility for the program was subsequently transferred from MAFF to the FSA upon its inception in April 2000. The NDNS programme itself consists of several surveys targeting specific age groups, which were conducted every 3 years in succession. The following surveys are available in electronic format and were used for the derivation of intake estimates of candy presented in this report: United Kingdom Data Archive (UKDA) for the NDNS: Adults Aged 16 to 64 years collected in 2000-2001 (NDNS 2000-2001) National Statistics, 2005), and the National Diet and Nutrition Survey: Young People aged 4 to 18 Years (NDNS 1997) (UKDA, 2001). Food records for individuals were selected using a stratified multi-stage random probability design, with sampling of private households throughout Great Britain using postal sectors (UKDA, 1995, 2001; Office for National Statistics, 2005) as the primary sampling unit.

NDNS data were collected from individuals as well as households *via* 7-day weighted dietary intake records throughout all 4 seasons of the year (4 fieldwork waves of 3 months duration), in order to address variability in eating behaviours due to seasonality. Dietary data were recorded by survey respondents or by parents or guardians in the case of the children's survey for the duration of the survey period. NDNS 2000-2001 contains 7-day weighed dietary records for more than 1,724 individuals aged 16 to 64, while NDNS 1997 contains 7-day records for approximately 1,700 youth aged 4 to 18 (UKDA, 1995, 2001; Office for National Statistics, 2005). Initial postal questionnaires and interviews were employed to identify eligible children, youth, or adults, respectively, for the surveys. Overall, response rates of 92%, and 73% were achieved; the maximum response rate (individuals agreeing to the initial dietary interview) from the eligible sample selected for participation in the survey were 80% and 61%, respectively, while only 64% and 47% of surveyed individuals completed a full dietary record (UKDA, 2001; Office for National Statistics, 2005).

The NDNS programme collects physiological, anthropometric and demographic information from individual survey participants, such as sex, age, measured height and weight (by the interviewer), blood analytes, and other variables useful in characterizing consumption in addition to collecting information on the types and quantities of foods being consumed. Further assessment of food intake based on consumption by specific population groups of interest

within the total surveyed samples was made possible by the inclusion of this information. In order to compensate for the potential under-representation of intakes from specific population groups resulting from sample variability due to differential sampling probabilities and differential non-response rates [particularly the lower response rate among males aged 15 to 18 years (UKDA, 2001)], sample weights were developed and incorporated with the youth survey (NDNS, 1997).

Estimates for the intake of gum or mints by the U.K. population were generated and collated by computer, using consumption data from individual dietary records, detailing food items ingested by each survey participant on each of the survey days. Estimates for the daily intake of gum or mints represent projected 7-day averages for each individual from Days 1 to 7 of NDNS data. The distribution from which mean and percentile intake estimates were produced was comprised of these average amounts. Mean and percentile estimates were generated using ratio estimation and nonparametric techniques, incorporating survey weights where appropriate in order to provide representative intakes for specific U.K. population groups. All-person intake refers to the estimated intake of gum or mints averaged over all individuals surveyed regardless of whether they consumed gum or mint products, and therefore includes "zero" consumers [those who reported no intake of gum or mints during the 7 survey days]. All-user intake refers to the estimated intake of gum or mints by those individuals consuming food products in which the use of gum or mints is either currently under consideration, hence the "all-user" designation. Individuals were considered users if they consumed a gum or mint product on one of the 7 survey days.

Table IX.c-1 Summary of the Estimated Daily Intake of Chewing Gum in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	Actual No. of Total Users	All-Person Consumption			All-Users Consumption		
			Mean (g)	Percentile (g)		Mean (g)	Percentile (g)	
				90	95		90	95
Young People	4-11	91	0.195	0	0.857*	2.10	4.86*	6.00*
Teenagers	12-18	108	0.516	0.571*	2.571*	3.25	6.86*	14.00*
Adults	19-64	67	0.070	0	0	1.71	2.86*	8.57*

*Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements. NA = Not Applicable (insufficient number of users to generate 90th and 95th percentile estimates). See Appendix C for copy of intake assessment.

Table IX.c-2 Summary of the Estimated Daily Intake of Mints in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	Actual No. of Total Users	All-Person Consumption			All-Users Consumption		
			Mean (g)	Percentile (g)		Mean (g)	Percentile (g)	
				90	95		90	95
Young People	4-11	100	0.315	0.143	2.14	3.12	7.71	10.4
Teenagers	12-18	55	0.265	0	1.71	3.67	7.29*	11.6*
Adults	19-64	55	0.086	0	0	2.58	5.74	10.9*

*Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements. Mean and 95th percentile intake estimates based on sample sizes of less than 30 and 160 respectively, may not be considered statistically reliable due to the limited sampling size (LSRO, 1995). See Appendix C for copy of intake assessment.

Table IX.c-1, and IX.c-2 summarizes the estimated total intake of gum and mints respectively (g/person/day) by the U.K. population groups. Teenagers were determined to have the greatest mean and 95th percentile all-user intakes of gum or mints on an absolute basis of the individual population groups, with values of 3.25 and 14 g/person/day, and 3.67 and 11.6 g/person/day respectively. Intakes in children and adults were comparable (Tables IX.c-1, and IX.c-2).

Table IX.c-3 Wrigley Marketing Data: Gum and Mint Consumption in the U.K.

Food Type	No. of Users	Mean	90th Percentile	95th Percentile
Gum	959	3.06	7.1	12.1
Mint	959	2.06	4.50	9.03

Mean and 95th percentile intake estimates based on sample sizes of less than 30 and 160, respectively, may not be considered statistically reliable due to the limited sampling size (LSRO, 1995). As such, the reliability of estimates for the intake of gum or mints based on the consumption of these foods may be questionable for certain individual population groups. As shown in tables IX.c-1 and IX.c-2, the statistical reliability of the estimated gum and mint intake may be unreliable for some population groups due to the small sample size of the users in these categories. To provide additional confidence in the estimates, Wrigley's compiled in-house marketing survey data for mint and gum consumption in the United Kingdom. The data represents the results of 7-day surveys, among a broad population range (ages 1 through 90+) and are expressed as total population mean, 90th and 95th percentile consumption. As shown in table IX.c-3 above, the estimated gum and mint consumption is comparable to those estimated using the NDNS databases.

Table IX.c-4 Summary of the Estimated Daily Intake of MBSE under the Proposed use of MBSE in Chewing Gum in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	Actual No. of Total Users	All-Person Consumption			All-Users Consumption		
			Mean (mg)	Percentile (mg)		Mean (mg)	Percentile (mg)	
				90	95		90	95
Young People	4-11	91	0.4	0	1.7*	4.2	9.7*	12.0*
Teenagers	12-18	108	1.0	1.1*	5.1*	6.5	13.7*	28.0*
Adults	19-64	67	0.1	0	0	3.4	5.7*	17.1*

*Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements. Mean and 95th percentile intake estimates based on sample sizes of less than 30 and 160 respectively, may not be considered statistically reliable due to the limited sampling size (LSRO, 1995). See Appendix C for copy of intake assessment.

Table IX.c-5 Summary of the Estimated Daily Intake of MBSE Under the Proposed use of MBSE in Mints in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	Actual No. of Total Users	All-Person Consumption			All-Users Consumption		
			Mean (mg)	Percentile (mg)		Mean (mg)	Percentile (mg)	
				90	95		90	95
Young People	4-11	100	0.63	0.3	4.3	6.2	15.4	20.8
Teenagers	12-18	55	0.53	0	3.4	7.3	14.6*	23.2*
Adults	19-64	55	0.17	0	0	5.2	11.5*	21.8*

*Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements. Mean and 95th percentile intake estimates based on sample sizes of less than 30 and 160 respectively, may not be considered statistically reliable due to the limited sampling size (LSRO, 1995). See Appendix C for copy of intake assessment.

Based on a proposed maximum use level of MBSE in mints and gum at concentration of 0.2% of the finished product, estimates of exposure to MBSE were determined. On an absolute basis, highest exposure to MBSE was observed in teenagers with 95th percentile estimates of 28 and 23 mg/person per day for gum and mint consumption (Tables IX.c-4 and IX.c-5). On an mg/kg basis, exposure to MBSE in the diet was highest in children at 0.6 and 1.04 mg/kg body weight per day (Tables IX.c-6 and IX.c-7).

Table IX.c-6 Summary of the Estimated Daily Per Kg Body Weight Intake of MBSE under the Proposed use of MBSE in Chewing Gum in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	Actual No. of Total Users	All-Person Consumption			All-Users Consumption		
			Mean (mg)	Percentile (mg)		Mean (mg)	Percentile (mg)	
				90	95		90	95
Young People	4-11	91	0.02	0.00	0.09*	0.21	0.49*	0.60*
Teenagers	12-18	108	0.02	0.02*	0.10*	0.13	0.27*	0.56*
Adults	19-64	67	0.00	0.00	0.00	0.05	0.08*	0.24*

*Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements. Mean and 95th percentile intake estimates based on sample sizes of less than 30 and 160 respectively, may not be considered statistically reliable due to the limited sampling size (LSRO, 1995). #Body weights of 20, 50 and 70 kg were used to derive the mg/kg exposures from tables IX.c-4 and IX.c-5. See Appendix C for copy of intake assessment.

Table IX.c-7 Summary of the Estimated Daily Per Kg Body Weight Intake of MBSE Under the Proposed use of MBSE in Mints in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	Actual No. of Total Users	All-Person Consumption			All-Users Consumption		
			Mean (mg)	Percentile (mg)		Mean (mg)	Percentile (mg)	
				90	95		90	95
Young People	4-11	100	0.03	0.01	0.22	0.31	0.77	1.04
Teenagers	12-18	55	0.01	0.00	0.07	0.15	0.29*	0.46*
Adults	19-64	55	0.00	0.00	0.00	0.07	0.16*	0.31*

*Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements. Mean and 95th percentile intake estimates based on sample sizes of less than 30 and 160 respectively, may not be considered statistically reliable due to the limited sampling size (LSRO, 1995). #Body weights of 20, 50 and 70 kg were used to derive the mg/kg exposures from Tables IX.c-4 and IX.c-5. See Appendix C for copy of intake assessment.

IX.d MBSE Release from Chewing Gum

Studies also were conducted to determine the quantity of MBSE that is released from the product during chewing. MBSE was incorporated into the centre or the outer coating of the gum at the same level as used in the stability studies (1 mg/stick of gum). Each piece of gum was chewed by 6 subjects for a period of 6, 12, and 20 minutes and the amount of MBSE remaining in the gum was quantitated. Magnolol and honokiol are very hydrophobic and as such very little MBSE was found to be released from the gum during chewing when the extract was incorporated into the centre of the product (Figure/Table IX.d-1). When incorporated into the outer coating, up to 50% of the MBSE was released over a 20 minute chewing interval. Based on these results, Wrigley's has decided to incorporate the MBSE into the outer coating of their gum products. In addition, it should also be noted that based on these results, the estimated exposure to MBSE following consumption at the proposed intakes in chewing gum would be reduced by approximately 50% of the incorporation levels.

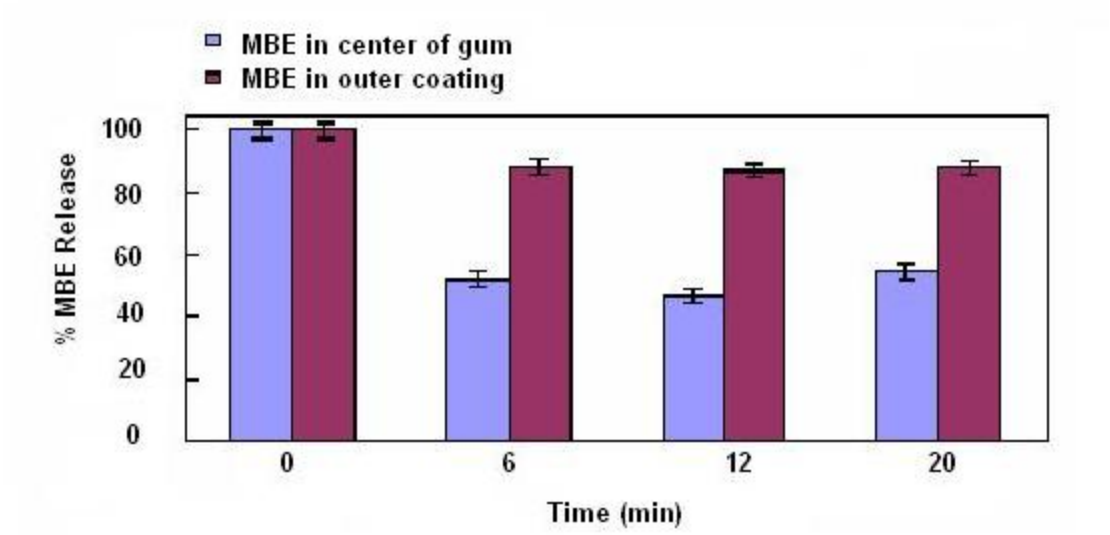


Figure IX.d-1 Release of MBSE from Chewing Gum.
The hydrophobicity of magnolol and honokiol causes limited release from chewing gum when the extract was incorporated into the centre of the product.

Table IX.d-1 Release of MBSE from Chewing Gum

Time (minutes)	% MBSE retained in gum when added to gum coating	%MBSE retained in gum when added to gum pellet
0	100 ± 2.3	100 ± 2.3
6	52.3 ± 2.4	88.5 ± 2.3
12	46.5 ± 2.7	87.1 ± 2.3
20	54.7 ± 2.3	88.0 ± 2.3

IX.e Conclusion

Consumption data and information pertaining to the individual proposed food-uses of MBSE were used to estimate the all-person and all-user intakes of MBSE for specific demographic groups and for the total U.K. population. This type of intake methodology is generally considered to be "worst case" as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use, which would result in an overestimation of MBSE exposure as the ingredient will be restricted to use only in Wrigley products. In addition, due to the hydrophobic nature of the major constituents of MBSE (magnolol and honokiol), exposure to these constituents from use in gum is expected to be overestimated by approximately 50%.

In summary, the highest exposures to MBSE under the proposed food uses were estimated to occur in teenagers, where mean and 95th percentile exposures were determined to be 6.5 and

28 mg/person/day (0.13 and 0.56 mg/kg body weight) for gum consumption and 7.3 and 23.2 mg/person/day (0.15 and 0.46 mg/kg body weight) for mint consumption respectively. On an mg/kg basis, highest exposures were estimated from children, where the intake of MBSE was determined to be 0.21 and 0.60 mg/kg body weight in mean and 95th percentile gum users respectively; corresponding exposure to MBSE from mint consumption in these users was 0.22 and 1.04 mg/kg body weight per person per day. Although combined exposures to both mint and gum under the proposed uses were not conducted, the estimated exposures in users of both products can be considered on an additive basis. Typically, the summation of percentile exposures from individual food is not recommended since these estimates are not considered representative of any one consumer (DiNovi and Kuznesof, 1995); however, for conservative reasons a consideration of combined/additive exposure to MBSE under the proposed food uses is presented. On an absolute basis, combined exposure to MBSE from gum and mints would be approximately 14 and 50 mg/person/day for mean and 95th percentile highest users (teenagers). Highest combined exposures on an mg/kg basis would be 0.5 and 1.64 mg/kg body weight per day for mean and 95th percentile child users.

X INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO MAGNOLIA BARK EXTRACT

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to previous human exposure to the novel food:

- "Is there information from previous direct, indirect, intended or unintended human exposure to the novel food or its source which is relevant to the EU situation with respect to production, preparation, population, lifestyles and intakes?"

- "Is there information to demonstrate that exposure to the novel food is unlikely to give rise to mitochondrial, toxicological and/or allergenicity problems?"

These questions have been addressed collectively in Sections X.a and X.b.

X.a Natural Occurrence and History of Use of MBSE in the Diet

Exposure to magnolol and honokiol, the principal constituents of MBSE from the consumption of various traditional European diets is not expected. The use of MBSE as described herein has been determined to be Generally Recognized as Safe in the United States, and gum and mint products produced by Wrigley have been introduced to the U.S. marketplace. There have been no reported incidences of adverse effects associated with the use of these products.

X.b Potential Allergenicity Concerns

MBSE is isolated from the bark of *M. officinalis* using supercritical carbon dioxide extraction and therefore does not contain protein; as such, allergy concerns are not warranted.

XI NUTRITIONAL INFORMATION ON MBSE

Based on the SCF guidelines, the following question must be answered in the affirmative to ensure sufficient nutritional information pertaining to the novel food:

- "Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?"

This question has been addressed in Section XI.a.

XI.a Nutritional Benefits of MBSE

The proposed use of MBSE in mints and gum is to provide breath freshening characteristics to these products. As described in section IX.c, under the proposed food uses of MBSE, exposure to the ingredient is limited, and the product is not expected to have a nutritional impact on the diet.

XIII TOXICOLOGICAL INFORMATION ON MBSE

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient toxicological information pertaining to the novel food:

“Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?”

“Compared to the traditional counterpart, does the novel food contain any new toxicants or changed levels of existing toxicants?”

or

“Is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?”

“Is there information which suggests that the novel food might pose an allergenic risk to humans?”

These questions have been addressed collectively in Section XIII.a.

XIII.a Safety Studies Conducted with MBSE

XIII.a.1 Sub-Chronic Toxicity

A 21-day toxicity study was conducted on Wrigley’s MBSE in male and female Sprague-Dawley rats (Liu *et al.*, 2007). The experiment was a pilot dose-ranging study used to determine the optimum dose for the subsequent 90-day subchronic study and was conducted in-line with U.S. FDA and Organization for Economic Cooperation and Development (OECD) guidelines pertaining to Good Laboratory Practice (GLP), and was performed in accordance with U.S. FDA Redbook 2000 guidelines for the safety assessment of food ingredients (U.S. FDA, 2000). The MBSE test article used for the experiment contained 94% magnolol and 2.0% honokiol[3]. Male and female Sprague-Dawley rats, aged 6 to 7 weeks, weighing between 100 to 120 grams were used. All animals were subjected to physical examination for clinical signs of ill health and were observed within 7 days of arrival. Following acclimatization for 7 days, animals were re-examined and weighed. In the 21-day study, animals were randomly assigned to 5 groups of 5 males and 5 females on the basis of body weight. In the 90-day study, rats whose weight variation exceeded 2 standard deviations of the mean weight of each sex were eliminated from study selection. Rats were randomly assigned to 4 groups of 20 males and 20 females on the basis of body weight. In both studies, initial mean body weights and weight distributions were

[3] The product used in the 21-day and 90-day studies conformed to the product specifications listed in Table I.e-1.

similar in each group. Animals were individually housed in suspended stainless steel, open-mesh cages in environmentally controlled rooms (23 ± 2°C, 12-hours light/dark cycle, 30 to 70% relative humidity, ventilation frequency of 10 to 15 times/hour).

Animals were observed twice daily for clinical signs and mortality. Food spillage was also assessed twice daily by visual inspection of the amount of food droppings beneath each cage. In the 21-day study, body weights were measured at the start of treatment (day 0), on days 1, 4, 8, 10, 14, 16, 20, and 21, and at sacrifice following fasting. Food consumption measurements were taken on day 1, over days 2 to 3 and over days 4 to 7 in the first week of the study. Thereafter, food consumption was measured weekly, and the total food consumption, food utilization in each week, and the total food utilization (weight gain per 100 g food consumed) was calculated at the end of the study.

In the 90-day study, body weights were measured prior to treatment, weekly during treatment, and at sacrifice after fasting (Liu *et al*., 2007). Food consumption was determined weekly and total food consumption for each animal was calculated at the end of the study. Food utilization in each week and total food utilization during the study were also determined. Prior to treatment, all animals were subjected to ophthalmic examination using an indirect ophthalmoscope following dilation of eyes with tropicamide. Animals in the control and high-dose groups also underwent ophthalmic examinations at week 13.

For the 21 day study animals were randomized to one of 5 treatment groups receiving MBSE in the diet such that groups would consume theoretical doses of 0, 60, 120, 240, or 480 mg/kg body weight of MBSE extract per day. Haematology measurements included: red blood cell count (RBC), haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin, platelet count, white blood cell count (WBC), differential blood cell count, and reticulocyte count. A bone marrow smear was also taken from the sternum and was fixed, stained and examined for the following parameters: myeloblasts, promyelocytes, myelocytes, metamyelocytes, stab nucleus neutrophils, lobocytes, eosinophils, basophils, proerythroblasts, early normoblasts, intermediate normoblasts, late normoblasts, lymphocytes, plasma cells, monocytes, and myeloid-to-erythroid ratio. Haematology parameters were measured using an automated hematoanalyzer (MEK-7222J/K, NIHON KOHDEN).

To assess coagulation, activated partial thromboplastin time was determined from plasma, using sodium citrate as an anticoagulant. Coagulation parameters were analyzed by an automated hematocoagulation parameter (MC-4000 PLUS, Medicine Devices GmbH & Co. KG).

For serum chemistry analysis, blood was centrifuged and serum was separated. Serum chemistry parameters included: glucose, urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), aspartate aminotransferase (AST),

total protein, albumin, cholesterol, triglycerides, total bilirubin, alkaline phosphatase (ALP), calcium, inorganic phosphorus, sodium, potassium, and chloride. Parameters were analyzed using an automatic clinical analyzer (7080, Hitachi High-Technologies Corporation).

Urine was collected for 16 to 18 hours prior to blood collection, and was assessed for: appearance/colour, specific gravity, pH, protein, glucose, ketones, bilirubin, blood, urobilinogen, nitrite, and leukocytes. Urinalysis was conducted using a urine analyzer (Combi SCAN 100, analyticon Biotechnologies AG Germany). Urine chemistry measurements included: calcium, sodium, potassium, phosphorus, and chloride. These were measured using an autoanalyzer (7080, Hitachi High-Technologies Corporation).

All rats were humanely euthanized following blood sample collection, and a complete necropsy was performed. Organ weights were obtained for the adrenal glands (2), brain, epididymis (2), heart, kidneys (2), liver, spleen, ovaries (2), stomach (without contents), testes (2), thymus, thyroid (2 lobes) with parathyroid, and uterus. Paired organs were weighed together. Organ-to-body weight ratios (relative weight) were also calculated. In addition to the above-mentioned organs, several tissues were sampled and fixed in 10% neutral-buffered formalin. These included the aorta, cecum, colon, cervix, duodenum, oesophagus, eyes (2), femur with bone marrow, ileum, jejunum, lacrimal gland, lung, lymph node, mammary gland, nasal turbinates, pancreas, Peyer's patches, pituitary gland, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles (2), skeletal muscle (thigh), skin, spinal cord, sternum with bone marrow, trachea, urinary bladder, vagina, and Zymbal's gland.

All stored organs and tissues from each animal in the control, 240 mg/kg body weight/day, and 480 mg/kg body weight/day groups were embedded in paraffin, sectioned, stained with haematoxylin and eosin, and subjected to microscopic examination. Macroscopic lesions observed at necropsy were also examined from each animal in other dose groups. Treatment with MBSE did not affect mortality or clinical signs. No differences in body weight, body weight gain, or food utilization were observed. A decrease in food consumption was reported in females of the high-dose group compared to control animals at week 3, but no difference in total food consumption was observed. No statistically significant differences in the majority of the haematology parameters were observed between the treatment and control groups. Compared to male rats in the control group, the red blood cell (RBC) value was significantly lower ($p<0.01$) in the 60 mg/kg body weight/day group, and haematocrit values were significantly ($p<0.05$) higher in the 120 mg/kg body weight/day group. In female rats, haematocrit was significantly ($p<0.05$) higher in the 240 mg/kg body weight/day group compared to control animals, and the mean corpuscular haemoglobin value in the high-dose group was significantly ($p<0.01$) higher than the control group. These haematological changes were not dose responsive, not consistent between sexes and were of low magnitude (8 to 15%), and therefore were not considered to be of biological relevance.

Bone marrow evaluation revealed no statistically significant differences. Additionally, no significant differences in activated partial thromboplastin time, a measure of coagulation, were reported between MBSE-treated and control groups.

Clinical chemistry analysis indicated no statistically significant differences for all parameters measured between male treatment and control groups and for the majority of parameters in female rats. BUN levels were significantly higher (+25%; $p<0.05$) in the 120 mg/kg body weight/day females but no significant differences were observed in the higher dose groups compared to controls. Urinalysis was unremarkable with the exception of sodium values, which were significantly higher (+80%; $p<0.05$) in the 120 mg/kg body weight/day male group compared to controls. The increase in urine sodium is not toxicologically relevant since the effect was not observed in the higher doses of 240 and 480 mg/kg body weight and similar effects did not occur in the female rats.

Absolute organ weights and organ-to-body weight ratios (relative weight) in treated groups were not significantly different from control groups with 2 exceptions. Absolute and relative thyroid weights (+38 and 43%; $p<0.05$ respectively), as well as relative kidney weight, were slightly but significantly increased (+16%; $p<0.05$) for females of the high-dose group, and relative spleen weight was slightly but significantly increased (+21%; $p<0.05$) for males of the 60 mg/kg body weight/day group compared to their respective controls. Organ weights were within the historical range of control weights and were not accompanied by evidence of clinical, gross or microscopic pathological effects, indicating that organ weight differences were not toxicologically relevant. Additionally, no biologically significant changes attributable to the test article were observed in RBC and WBC counts, which are indicators of spleen function. No significant differences in macroscopic or microscopic pathology findings were reported.

The no-observed-adverse-effect level (NOAEL) for this study is 480 mg/kg body weight/day, the highest dose tested.

For the 90-day subchronic toxicity study the MBSE test article used for the experiment was incorporated into the diet in an identical manner to that used in the 21-day pilot study. The formulated feed diet used during the first week was based on data for food consumption from the 21-day pilot study. All subsequent formulations were based on food consumption per 100 g body weight during the previous week's feeding interval. Both stability and homogeneity studies were conducted prior to the experiment indicating that MBSE is stable in feed for at least 14 days, and is evenly distributed throughout the feed supply.

Groups of 20 male and female Sprague-Dawley rats (100 to 120 g) were randomized to 1 of 4 groups receiving MBSE formulated in the feed, resulting in MBSE intakes of 0, 60, 120, and

240 mg/kg body weight of MBSE per day for both males and females.[4] Animals were observed twice daily for abnormalities, physical appearance and mortality. Food inspection was observed twice daily by visual inspection of the amount of food droppings beneath the cage of each animal. Animal weights were measured prior to treatment, weekly thereafter and at termination after fasting. Food consumption for each animal was determined weekly and at study termination. In addition, food utilization (weight gain per 100 g food consumption) in each week and total food utilization were also calculated. Ophthalmic observations were made in all animals prior to treatment and in control and high dose animals at week 13. Standard blood haematological and biochemical analysis, including a full urinalysis was conducted as reported above. In addition, bone marrow smears sampled from the sternum of control and high-dose animals were also prepared. On day 91 all animals were euthanized and a complete gross examination was performed as per the 21-day study.

No mortality, ophthalmic abnormalities, or treatment-related adverse clinical reactions were observed. Body weights did not significantly differ between the female treatment groups and the control group, but males in the 120 mg/kg body weight group had significantly higher body weights than the control group at week 3. Significantly lower weight gains were observed in females of the 120 mg/kg body weight group compared to the control group at week 4, and of the 240 mg/kg body weight group compared to the control group at week 7. In males, significantly higher weight gains in the 120 and 240 mg/kg body weight groups were observed during week 2 compared to control animals. Differences in body weight and body weight gain were not considered to be toxicologically significant as they did not occur in a dose-related manner and the 3 treated groups had comparable total weight gains to the control group. Food consumption and food utilization did not differ among groups in females, but consumption was significantly lower compared to controls in males in the 240 mg/kg body weight group in week 1 and significantly higher in males in the 60 and 120 mg/kg body weight groups in weeks 5 and 12, respectively. Total food consumption was significantly higher in the 120 mg/kg body weight group in comparison to the control group. Food utilization in males from the 120 and 240 mg/kg body weight groups in week 2 was significantly higher while food utilization in the 120 mg/kg body weight group in week 12 was significantly lower compared to the control group. No difference in total food utilization was observed between treated and control groups. Variations in food consumption and utilization were not considered to be treatment related as there was no dose-response relationship and values were within the laboratory's historical normal range of controls.

No statistically significant differences between treatment and control groups were observed for all haematological, bone marrow and coagulation parameters. Clinical chemistry measurements revealed no statistically significant differences for all parameters measured

[4] In consideration of animal welfare, the top dose for the 90-day study (240 mg/kg bw) was chosen on the basis of the size of the safety margin in comparison likely human exposure level.

between male treatment groups and the control group and for the majority of parameters in female rats. Blood total bilirubin (TBILL) and sodium (BNA) values in all three treatment groups were significantly ($p<0.01$) higher than those in the control group; however, these changes were modest (20 to 30% for TBILL and 5% for BNA), not dose responsive, only occurred in one sex and the absolute values were within the laboratory's historical normal range of controls. Thus, these statistical differences were not considered to be biologically meaningful. The only significant differences for urinalysis were lower potassium levels in the female 60 mg/kg body weight group as well as sodium and chloride values in the male 60 mg/kg body weight group. However, these changes did not occur in a dose-related manner, and were not observed in both sexes; therefore they were not considered to be biologically relevant. For absolute and relative organ weights, no significant differences were observed for any treatment group relative to controls in either sex. Furthermore, no treatment related effects were reported for clinical or gross pathology. Microscopic examination revealed slight fatty degeneration and sporadic focal necrosis of the liver, focal necrosis in the heart, and focal glomerulus pyknosis, but these were observed at similar frequencies in the control and treatment animals and were within the range of normal background lesions. Therefore, the effects were considered incidental and reflected the usual individual variability without any relationship to treatment.

Based on a lack of significant toxicological findings at all doses in the 90-day study, the NOAEL is 240 mg MBSE/kg body weight, the highest dose tested.

XIII.a.2 Mutagenicity and Genotoxicity

XIII.a.2.1 In Vitro

The mutagenicity of Wrigley's MBSE was evaluated by Li *et al.* (2007) in *Salmonella typhimurium* (*S. typhimurium*) strains TA98, TA100, TA1535 and TA1537 and in the *Escherichia coli* (*E. coli*) mutant, WP2 uvrA[5]. The magnolia bark extract was assayed by HPLC to contain 94% magnolol and 2.0% honokiol and was the same MBSE lot used in the sub-chronic toxicity studies reviewed above (Section 6.2.2). A preliminary cytotoxicity study was conducted in all strains using three plates per dose (ranging from 18.75 to 5,000 µg/plate) of MBSE in the absence of metabolic activation. The Ames assay (plate incorporation method) was performed with or without metabolic activation by S-9. The test sample [MBSE dissolved in dimethyl sulfoxide (DMSO)] was assayed in triplicate at 5 concentrations (18.75, 37.5, 75, 150, and 300 µg/plate), and 2 independent experiments were performed for each bacterial strain. Furylfuramide, sodium azide, and 9-aminoacridine were used as positive controls in the absence of metabolic activation, and benzo-a-pyrene, and 2-aminoanthracene were used as

[5] The study was conducted in-line with the standards of U.S. FDA GLP regulations and OECD Principles of GLP, and the methodology was also consistent with the OECD Guideline for Testing of Chemicals-471, Bacterial Reverse Mutation Test (1997), and the FDA Redbook, 2000, Toxicological Principles for the Safety Assessment of Food Ingredients, IV.C.1.a. Bacterial Reverse Mutation Test

positive controls for treatments in the presence of metabolic activation. DMSO was used for the negative controls.

In the preliminary toxicity and range-finding study, evidence of cytotoxicity (reduced rate of spontaneously occurring colonies and visible thinning of the bacterial lawn) was observed at concentrations of ≥75 µg/plate in *S. typhimurium* TA 1535 and TA 1537, ≥150 µg/plate in *S. typhimurium* TA 98, and ≥300 µg/plate in *S. typhimurium* TA100 and *E. coli* WP2*uvr*A. Based on these results, a range of concentrations from 18.75 to 300 µg/plate was selected for the bacterial reverse mutation test. In the mutagenicity study, MBSE did not increase the mean number of revertants per plate of any *S. typhimurium* strain or the *E. coli* strain, with or without metabolic activation, in comparison to the spontaneous reversion rate in the negative control. A reduction in the background bacterial lawn was observed at 300 µg/plate in *S. typhimurium* strains TA98 and TA100 and in *E. coli* WP2 *uvr*A. The number of revertants could not be counted for *S. typhimurium* strains TA1535 and TA1537 at concentrations of 150 and 300 µg/plate due to excessive cytotoxicity, but a lack of increase in the mean number of revertants per plate compared to negative control was observed for the remaining three concentrations, the highest of which produced evidence of cytotoxicity. The authors concluded that MBSE was not mutagenic in the above *S. typhimurium* and *E. coli* strains under the experimental conditions used in the study.

The same MBSE lot has also been investigated in two *in vitro* chromosomal aberration assays conducted in Chinese hamster ovary (CHO) cells or V79 cells derived from Chinese hamster lung tissue (Zhang *et al*., 2008). As above, these studies were also conducted in accordance with GLP principles and using methodology consistent with OECD and US-FDA Redbook 2000 guidelines for *in vitro* chromosomal aberration assays. Three experiments were conducted in CHO cells. Cells were exposed to 2.2, 7, 20, 25, 30, 35, 40, 50, and 60 µg MBSE/mL in experiment a, 1, 1.25, 2.5, 5, 6, 7, 8, 9, 10, and 15 µg MBSE/mL in experiment b, and 0.2, 0.6, 1.7, 5, 10, 15, 20, 25, 30, 35, and 40 µg MBSE/mL in experiment c. Experiments a and c were conducted in the absence of metabolic activation, while experiment b was conducted with metabolic activation by S-9. DMSO was used as a negative control, while cyclophosphamide and mitomycin C were used as positive controls for experiments conducted with and without metabolic activation, respectively. In experiments a and b, cells were incubated for 3 hours, followed by a wash step and further incubation for 15 hours in fresh culture medium. In experiment c, the duration of treatment was 18 hours without washing. Experiments were conducted in duplicate.

In experiment a, only the cells from the 0, 2.2, 7, 20 and 30 µg/mL groups were scored for chromosomal aberrations, as cytotoxicity was observed at higher dose levels. One aberrant chromosome was observed in a 2.2 µg/mL culture (0.5% of cells) and 3 aberrant chromosomes were found in 30 µg/mL cultures (1.5% of cells). These levels were consistent with spontaneous mutation. In experiment b, cells treated with 0, 1.25, 2.5, and 7 µg/mL were

examined for chromosomal aberrations. Only 1 chromatid break and 1 chromosome break were observed in the 7 µg/mL cultures. In experiment 3, cells treated with 0, 0.6, 1.7, 5, and 15 µg/mL for 18 hours were examined for chromosomal aberrations, and only 1 isochromatid break and 1 ring chromosome were observed from cultures treated with 0.6 µg/mL. Incidences of numerical chromosome changes in all 3 experiments were comparable to those from historical negative controls for this cell line. The positive control substances induced significant numbers of cells with chromosomal aberrations with similar values as those from historical control data. Treatment with DMSO only (negative control) also resulted in chromosomal aberration incidences similar to those from historical negative control data.

Based on the results of a preliminary dose range-finding test Chinese hamster V79 cells, 52 and 59 µg/mL were selected as the highest doses of MBSE to be utilized in the chromosomal aberration assay in the absence and presence of S9, respectively. Doses were 6.5, 13, 26, and 52 µg/mL without S9, and 7.5, 15, 30, and 59 µg/mL with S9. In the chromosomal aberration assay, cells were exposed to MBSE with or without S9 mix for 6 hours, after which cells were cultured in fresh medium for an additional 18 h (37°C). Cells exposed to MBSE without S9 mix were also incubated for 24 h without washing. DMSO was used as a negative control, while 4 µg/mL cyclophosphamide and 0.4 µg/mL mitomycin C were used as positive controls for experiments conducted with and without metabolic activation, respectively.

For cells exposed to solvent control only, specific chromosomal aberrations were detected in 2.0 and 2.5% of cells incubated in the absence of metabolic activation for 6 and 24 hours, respectively, and in 2.5% of cells incubated in the presence of metabolic activation for 6 hours. Rates of chromosomal aberrations in cells treated with MAGNOLIA BARK EXTRACT were not significantly different from negative control rates at all dose levels tested. Treatment with positive control substances resulted in high incidences of chromosomal aberrations.

XIII.a.2.2 In Vivo

In addition to the *in vitro* mutagenicity assays summarized above, Li *et al.* (2007) also conducted a micronucleus assay in male and female Swiss Albino (CD-1), mice 7 to 9 weeks of age[6]. In order to assess the toxicity of MBSE in mice and to select dose levels for the micronucleus test, a dose range-finding study was conducted in 5 male and 5 female mice. Animals were administered 2,500 mg of MBSE/kg body weight suspended in 0.5% aqueous carboxymethyl cellulose by oral gavage, and observed for 14 days. No mortality or evidence of toxicity was observed. Therefore, based on the results of the dose range-finding study, 2,500 mg/kg body weight was selected as the high dose for the micronucleus test, while 1,250

[6] The study was conducted to the standards of U.S. FDA GLP regulations and OECD Principles of GLP. The methodology was consistent with the OECD Guideline for Testing of Chemicals, Mammalian Erythrocyte Micronucleus Test, 1997, and FDA Redbook, 2000, Toxicological Principles for the Safety Assessment of Food Ingredients, in vivo Mammalian Erythrocyte Micronucleus Test.

and 625 mg/kg body weight were selected as mid- and low-dose levels. Animals were randomly allocated into one of five groups (5/sex/group): negative control (vehicle) group, 625 mg/kg body weight (low-dose group), 1,250 mg/kg body weight (mid-dose group), 2,500 mg/kg body weight (high-dose group), and positive control (40 mg cyclophosphamide/kg body weight) group. Animals were administered vehicle, positive control, or various doses of MBSE twice over an interval of 24 hours by oral gavage. Following dosing, animals were examined regularly for mortality and clinical signs of toxicity throughout the treatment period. Mice were euthanized by carbon dioxide asphyxiation 24 or 48 hours after the last treatment.

No mortalities were recorded, and gross necropsy revealed no macroscopic findings. The proportion of immature to total [immature + mature (normochromatic, NCE)] erythrocytes was not affected by MBSE administration, and no statistically significant increase in the number of micronucleated PCEs was observed in any of the MBSE-treated groups compared to the negative control group at either time point. As expected, the positive control substance induced a marked and statistically significant increase in the number of PCEs with micronuclei.

XIII.a.3 Human Studies

A two-part randomized, double-blind four-way crossover design clinical study was performed by Wrigley in order to measure the changes in hedonic oral odour resulting from consumption of sugar-free mints containing MBSE. Four test articles were used in the assessment: Peppermint mints, peppermint mints with 0.2% MBSE, peppermint mints with 0.2% MBSE and 0.2% sodium laurel sulfate (SLS), and an untreated control. Sixty-two healthy subjects (21 male/41 female) participated in the study, and were provided standard oral hygiene products for use during the trial in order to ensure routine oral hygiene procedures were the same amongst all participants. Use of all other oral hygiene products was restricted during the study, including mouthwashes, chewing gum and/or medicated lozenges, breath mints, or flavoured dental floss. The consumption of spicy or odiferous foods and alcohol was restricted for 2 days prior to each evaluation day. Subjects were also restricted from any form of oral hygiene, smoking, or consumption of dairy products after 10:00 pm on evenings prior to each evaluation day. Consumption of any food or drink and smoking activity or use of any oral hygiene products was restricted after 12 pm on evenings prior to part I of the evaluation until after the evaluation had been completed (A copy of the study report is provided in Appendix B).

The evaluations were completed as follows: following compliance checks, the study participants were randomized into 1 of the 4 treatment groups. Subjects in the three test article groups were provided with 1 mint and were instructed to dissolve the mint in their mouth without chewing within 20 minutes. Participants randomized into the untreated control group did not consume any type of product. Odour evaluations were performed 20, 50, 80, 110, and 180 minutes after consumption of the product by trained and experienced odour judges. The judges evaluated breath odour on a scale of 1 to 9, with 1 being “Most Pleasant” and 9 being “Most Unpleasant”. This procedure was completed 4 times, with at least 2 days between treatment days. During

part II, subjects were provided with a breakfast consisting of and egg and an English muffin upon arrival at the test site, and instructed to brush their teeth with water using their assigned toothbrush following consumption of the meal. Subjects were randomized to 1 of 4 treatment groups, and odour assessments were performed at 2, 3, and 4 hours after treatment. This procedure was also completed four times, with a minimum of 1 day between treatment days. The authors stated that no serious adverse events were observed during either trial indicating that adverse irritant effects on the oral mucosa was not noted; however, one subject reported a headache that study examiners determined may be related to use of the test product. The treatments were well tolerated otherwise, and overall a reduction in oral malodour was reported.

Wrigley also conducted two randomized, single-blind, crossover-design studies at the University of Illinois in Chicago in order to evaluate the effect of chewing gums and compressed mints containing MBSE on the reduction oral malodour (bacterial H_2S production) (Wrigley's, 2005b). In the first study, healthy subjects (n=15) arrived at the test site without having eaten food or brushed their teeth (or used any other oral hygiene product) after midnight on the day of the appointment, and un-stimulated whole saliva samples were collected using the drool method. Subjects were then instructed to dissolve 3 mints on their tongue, without chewing. The subjects received 1 of 5 treatments at each visit: Flavourless control candy, peppermint candy, flavourless candy containing 4.2 mg MBSE, peppermint candy containing 4.2 mg MBSE, or Listerine mouthwash (positive control). Saliva samples were obtained after 20, 40, and 60 minutes for analysis. Total and H_2S-producing bacteria were determined for each sample. The second study was carried out under the same conditions; however, the test substances administered to the subjects were in the form of chewing gum, and consisted of the following: Flavourless control gum, flavoured gum, flavourless gum containing 2 mg MBSE, and flavoured gum containing 2 mg MBSE, gum base, or Listerine mouthwash (positive control). Subjects were given 2 pellets of chewing gum and were instructed to chew with their mouth closed for 15 minutes, and then expectorate the gum. The chewing was performed under supervision with the use of a metronome, in order to ensure 60 chews per minute from all study subjects (Study protocols are included in Appendix B).

The results obtained from these studies indicate that the use of flavoured compressed mints containing MBSE resulted in a significant reduction in H_2S-production compared to baseline. A letter from the study investigator is included in Appendix B indicating that the use/consumption of MBSE containing mints and gum did not result in any adverse effects in any of the study participants in either study.

XIII.a.4 Summary

Wrigley's MBSE ingredient is of low oral toxicity in rodents. Product specific (Wrigley's MBSE) toxicological studies conducted with test articles meeting product specifications, indicate no treatment-related adverse effects. A NOAEL of 480 mg/kg body weight was determined in the 21-day pilot study, and a NOAEL of 240 mg/kg body weight was established in the 90-day

subchronic toxicity study, the highest doses administered. Mutagenicity and genotoxicity studies indicate that MBSE is not mutagenic or genotoxic. Ames tests conducted in the presence and absence of metabolic activation were negative, and in mammalian cells MBSE was non-genotoxic in Chinese Hamster Ovary cells with and without metabolic activation. *In vivo*, MBSE is non-genotoxic, as no evidence of micronucleus induction was observed in Swiss Albino (CD-1) mice receiving MBSE at doses up to 2,500 mg/kg body weight (Liu *et al.*, 2007). Human studies conducted with mints and gum formulated with MBSE and representative of the proposed commercial use indicate theses products will be well tolerated.

XIII.b Studies Conducted with Magnolol, Honokiol and Crude Magnolia Bark Preparations

Historically, crude magnolia bark preparations have long been used as a component of traditional Asian remedies (Hattori *et al.*, 1986; Tsai and Chen, 1992; Wang *et al.*, 1992; Tsai *et al.*, 1995; Ogata *et al.*, 1997; Sarker, 1997; Hsieh *et al.*, 1998; Maruyama *et al.*, 1998). Therefore, the majority of studies identified in the literature investigating the properties of Magnolia bark examine the crude powdered bark, or extracts thereof. Thus, it should be noted that the test articles used in these studies are not representative of the Wrigley's MBSE since these products likely contain significant quantities of plant alkaloids and other constituents that are not present in Wrigley's product. Nevertheless, these studies are reviewed below for the completeness.

Studies on the metabolic fate of magnolol and honokiol were identified. These compounds are the principle ingredients of Wrigley's MBSE and therefore were considered directly relevant to a safety evaluation of the product.

XIII.b.1 Metabolic Fate of Magnolol and Honokiol

XIII.b.1.1 Pharmacokinetics

Tsai *et al.* (1994a) investigated the pharmacokinetics of honokiol in male Sprague-Dawley rats. Blood samples were collected by cardiopuncture at 2.5, 5, 10, 15, 20, 30, 45, 60, and 120 minutes following the intravenous (i.v.) bolus injection of 5 or 10 mg/kg body weight honokiol. Analysis of plasma concentrations revealed that the pharmacokinetics of honokiol followed that of a two-compartment model, with gradual elimination from a central compartment. Similar elimination half-lives ($t_{½}$) were determined for both the 5 and 10 mg/kg body weight doses, 49.22 and 56.24 minutes, respectively, and short terminal elimination rate constants suggest that honokiol is rapidly eliminated from the systemic circulation. The apparent total body clearance (CL) did not differ between groups, and the area under the curve (AUC) increased proportionally with an increase in the *i.v.* bolus dose from 58.87 to 133.89 µg min/mL, for the 5 and 10 mg/kg body weight doses, respectively. These results led the authors to conclude that honokiol exhibits linear pharmacokinetics.

The disposition of magnolol was investigated in a similar fashion after i.v. bolus dosing (5 mg/kg body weight) or i.v. infusion (76 µg/kg body weight/minute) in male albino rabbits (Tsai *et al.*, 1994b). Blood samples collected at various time-points up to 120 minutes post-administration were used to generate the pharmacokinetic profile. Using a two-compartment open model, no significant differences were found in t½ or CL between i.v. bolus or infusion; t½ was 14.56 and 15.71 minutes, respectively, while CL was 75.86 mL/minute/kg and 72.72 mL/minute/kg. Consistent with the results observed for honokiol in rats (Tsai *et al.*, 1994a) the authors reported that the pharmacokinetic profile of magnolol in rabbits is linear.

Tsai *et al.* (1996) also investigated the pharmacokinetics of magnolol in male Sprague-Dawley rats, following an i.v. bolus injection of 2, 5, or 10 mg/kg body weight. Blood sampling was conducted as described for Tsai *et al.* (1994a). Samples of brain were collected and analyzed. As in previous reports (Tsai *et al.*, 1994a,b), the plasma concentration time-profile for magnolol demonstrated a biphasic disposition, with an early distribution phase and a slower elimination phase, best represented by a two-compartment open model. No significant differences were observed for t½, CL, steady-state volume of distribution (Vd_{ss}), or mean residence time (MRT), with increasing doses of magnolol. The t½ following an i.v. bolus dose of 2, 5, or 10 mg/kg body weight were 54.15, 49.05, and 49.58 minutes, respectively. The AUC and area under the moment *vs.* time curve (AUMC) appeared to increase linearly in the tested range. The authors also reported that 10 minutes after administering 5 mg/kg body weight magnolol i.v., brain concentrations of magnolol were approximately 4 times the concentration in plasma, but were uniformly distributed between brain regions. Brain distribution of magnolol was not ascertained following a 2 or 10 mg/kg body weight dose. Based on the dose-related results, the authors concluded that the pharmacokinetics of magnolol in the rat is also linear (Tsai *et al.*, 1996).

These investigations by Tsai *et al.* (1994a,b, 1996) suggest that magnolol and honokiol have similar pharmacokinetic properties, with similar elimination half-lives and disposition in the rat following an i.v. bolus dose (Tsai *et al.*, 1994a, 1996). Species differences were apparent; the half-lives of magnolol in rats were approximately 3 times those observed in rabbits (Tsai *et al.*, 1994b). However, in all 3 investigations there was no significant difference in the half-lives of magnolol or honokiol with increasing dose, suggesting that the kinetics of these 2 compounds is linear (Tsai *et al.*, 1994a,b, 1996), and not saturated up to an i.v. dose of 10 mg/kg body weight.

XIII.b.1.2 Absorption, Metabolism, Distribution and Excretion

XIII.b.1.2.1 Animal Studies

A series of experiments published in the Japanese Journal of Pharmacognosy were conducted to determine the absorption, distribution, metabolism, and excretion of magnolol (Hattori *et al.*, 1984, 1986; Ma *et al.*, 1988). Hattori *et al.* (1984) investigated the metabolism of magnolol following oral administration to 4-week-old male Wistar rats. Ten rats were administered daily suspensions (2 mL) of 50 mg magnolol in Arabic gum for 6 days. Urine and faecal samples were collected and combined at 24-hour intervals for analysis of benzene-soluble metabolites. Benzene-soluble fractions of faecal metabolites were isolated by preparative HPLC and their structures determined by electron impact ionization mass spectrometry. Five different metabolites were recovered, including isomagnolol (5,5'-di(1-propen-1(E)-yl)-2,2'dihydroxybiphenyl), tetrahydromagnolol, 5-(1-propen-1(E)-yl)-5'-propyl –2,2'-dihydroxybiphenyl, 5-allyl-5'-propyl-2,2'-dihydroxybiphenyl, and, 5-allyl-5'-(1-propen-1(E)-yl)-2,2'-dihydroxybiphenyl. Thus, varying degrees of reduction may have occurred in the allyl side chains yielding dihydro and tetrahydro derivatives, while isomerisation of allyl substituents appeared to yield faecal metabolites with propenyl side chains. Approximately 22% of the administered dose was excreted in the faeces as magnolol, within 24 hours of the first dosing. During hours 48 to 72, the major metabolites isomagnolol and tetrahydromagnolol increased linearly, with a concomitant decrease in the excretion of magnolol. Excretion of isomagnolol became constant after 96 hours, while tetrahydromagnolol did not reach a maximum until 120 hours post-initial administration. Excreted amounts of the major metabolites were not further quantified. The elution pattern for urinary metabolites was similar to those identified faecally, though the retention times differed slightly for several of the peaks (Hattori *et al.*, 1984).

An *in vitro* investigation of the role of rat intestinal flora in the metabolism of magnolol was also conducted as part of the same experiment. Fresh rat faeces were anaerobically incubated at 37°C for 48 hours with 10 µg/mL magnolol. HPLC analysis of the metabolites revealed isomagnolol and magnolol as the predominant constituents, while no chromatographic peak was apparent for tetrahydromagnolol, suggesting that under the experimental conditions, the intestinal bacteria were capable of converting an allyl to a propenyl substituent, but were incapable of further reductions (Hattori *et al.*, 1984).

In a subsequent experiment, Hattori *et al.* (1986) examined the absorption, metabolism, and excretion of radiolabelled [ring-^{14}C]magnolol, following oral or intraperitoneal administration of single or repeated doses to male Wistar rats. In the single dose trial, a 1 mL suspension of [ring-^{14}C]magnolol (2 µCi) in 5% Arabic gum was administered orally or intraperitoneally to 4- or 8-week-old rats. Faeces, urine, and bile were collected at various intervals. Following oral administration, bile was collected from bile-duct-cannulated rats with or without ligation of the gastric pylorus. To determine the distribution of radioactivity in organs and biological fluids, an additional group of 4-week-old rats was orally administered 1.6 µCi of [ring-^{14}C]magnolol as

0.6 mL of a 5% Arabic-gum suspension. Animals were sacrificed at intervals from 15 minutes to 48 hours post-administration.

Using a liquid scintillation counter, maximum radioactivity was detected in the blood of rats orally administered 2 µCi of [ring-^{14}C]magnolol 15 minutes (1.3×10^4 dpm/mL) after dosing, suggesting that magnolol was rapidly absorbed from the gastrointestinal tract. Blood levels declined from 30 minutes to 4 hours, reached a second peak at 8 hours (3.0×10^3 dpm/mL) and declined thereafter, up to 48 hours. Radioactivity was predominantly observed in the gastrointestinal tract and liver, with 11% of the administered dose present in the liver at 15 minutes. Lesser amounts of radioactivity were also detected in the kidney and pancreas. Distribution of radioactivity in the organs followed a similar time course to that observed in the blood, with peaks observed in the liver, kidney, and pancreas at 15 minutes and 8 hours. By 48 hours post-administration only 2% of administered radioactivity was still detected in the organs. Radioactive constituents were rapidly excreted into the bile following intraperitoneal administration; 47% of radioactivity was accounted for within 2 hours of dosing, reaching 80% by 10 hours. Oral administration resulted in a gradual biliary excretion profile, with 47% of the administered dose of radioactivity excreted in the bile 10 hours after dosing. The excretory profile did not differ depending on the status of the gastric pylorus. The major route of elimination was *via* the faeces; faecal excretion accounted for 65 and 52% of the radioactivity 24 hours after oral and intraperitoneal administration, respectively, while 11 and 24%, respectively, were observed in the urine. Within 6 days, approximately 80% of the orally or intraperitoneally administered radiolabelled doses were recovered (Hattori *et al.*, 1986).

Faecal, urinary, or biliary samples, collected from 0 to 72 hours (in the former 2 cases) or 0 to 4 hours (in the latter) after oral administration were further separated into benzene-soluble, ethyl acetate-soluble, and water-soluble fractions for analysis of radioactive metabolites. The ethyl acetate and water-soluble fractions were treated with β-glucuronidase and arylsulfatase in the presence or absence of a β-glucuronidase inhibitor (D-saccharic acid-1,4, lactone). Free forms of magnolol, including the reduced and isomerised metabolites identified by Hattori *et al.* (1984), predominated in the faeces at 53%, with lesser amounts in the urine and bile, 8.8 and 7.0%, respectively. Glucuronic acid and sulfate conjugates represented only 6.2 and 5.7% of the radioactivity observed in faeces and urine, respectively, but glucuronides contributed 48.4% of the radioactivity present in the bile, suggesting that conjugates formed in the liver may be hydrolyzed to free forms by the intestinal microflora prior to excretion in the faeces (Hattori *et al.*, 1986). After treatment of the ethyl acetate-soluble fraction of the bile with β-glucuronidase, the major aglycone (contributing approximately 50% to the excreted biliary radioactivity) identified *via* HPLC was magnolol, and the glucuronide was therefore concluded to be magnolol-2-*O*-glucuronide. Large amounts of unidentified products were present in faeces, urine, and bile, at 39.8, 85.5, and 42.3%, respectively (Hattori *et al.*, 1986).

The authors reported that radioactivity in the blood samples (fractionated as described above, data not shown) consisted primarily of free forms of magnolol, magnolol-*O*-glucuronide, and unidentified compounds in the benzene-soluble, ethyl acetate-soluble, and water-soluble fractions, respectively (Hattori *et al.*, 1986).

The enterohepatic circulation and gastrointestinal excretion of [ring-^{14}C]magnolol was further investigated by Ma *et al.* (1988) using bile-duct-cannulated rats. Male Wistar rats were intercannulated such that bile from one rat (rat A) was infused into the duodenum of a recipient animal (rat B). Rat A was intravenously administered a suspension of 13 mg/kg body weight (2.05 mCi/mmol) [ring-^{14}C]magnolol in 5% Arabic gum. Samples of bile, blood, and urine were collected at intervals until 4 hours after dosing, at which point both animals were sacrificed for measurement of hepatic radioactivity. Blood levels of radioactivity reached a peak in rat B at 1 hour (0.01 µmol equivalence of magnolol/mL), and declined thereafter, while biliary levels increased from 0.5 hours to the time of sacrifice at 4 hours, with a maximum of 1.3 µmol equivalence of magnolol/mL. Hepatic radioactivity was 0.33 and 0.23 µmol equivalence of magnolol/g tissue in rats A and B, respectively. From these results, the authors concluded that magnolol and its metabolites undergo enterohepatic circulation, and have a high affinity for hepatic tissues (Ma *et al.*, 1988).

To examine gastrointestinal excretion an additional group of bile-duct-cannulated rats were intravenously administered [ring-^{14}C]magnolol. Bile was collected at intervals and rats were sacrificed at 30 minutes, 1 hour, and 4 hours following the i.v. dose (Ma *et al.*, 1988). Approximately 60% of the administered radioactivity was secreted in the bile within 4 hours after dosing. As demonstrated using whole-body autoradiography, the liver, lungs, and kidney were the sites of highest radioactivity.

Tsai *et al.* (1995) conducted a separate experiment to assess the glucuronidation of magnolol. Blood samples were collected *via* cardiopuncture 30 minutes following the oral administration of 20 mg/kg body weight magnolol to male Sprague-Dawley rats. Plasma samples were analyzed with and without incubation with 50 µL β-glucuronidase at 37°C for 1 hour, in order to assess the concentrations of the free and conjugated forms of magnolol, respectively. The concentration of free and conjugated magnolol in plasma samples were 0.16 and 1.79 µg/mL, respectively, suggesting that magnolol is highly glucuronidated 30 minutes after oral administration in rats. It was also reported that magnolol has low bioavailability (less than 10%) following oral administration, suggesting that magnolol undergoes extensive first-pass metabolism in the liver prior to systemic distribution (Tsai *et al.*, 1995).

XIII.b.1.2.2 Human Studies

The elimination of magnolol was investigated in subjects using the traditional herbal remedy Saiboku-To (Homma *et al.*, 1993a). Saiboku-To is composed of a mixture of 10 different herbal extracts including *Bupleurum falcatum* Linne, *Pinellia ternata* Breitenbach, *Poria cocos* Wolf,

Scutellaria baicalensis Georgi, *Zizyphus vulgaris* Lamark, *Panax ginseng* C.A. Meyer, *Magnolia officinalis*, *Glycyrrhiza glabra* L., *Perillae frutescens* Britton, and *Zingiber officinale* Roscoe, with *M. officinalis* contributing 8.8% of the mixture on a dry weight basis. Nine patients (5 males and 4 females) were administered 2.5 g Saiboku-To as a granular extract 3 times/day, 2 hours after meals, for a total daily dose of 7.5 g, including 3.15 mg/day magnolol. Twenty-four-hour urine samples were collected from 12 to 20 weeks following the initiation of treatment, and analyzed *via* HPLC for free and glucuronic acid conjugated magnolol. Conjugated magnolol concentrations were determined following pre-treatment of urine samples with β-D-glucuronidase. Patients' clinical status was evaluated for 52 weeks prior to and following the onset of Saiboku-To use. The recovery of total and conjugated magnolol was calculated from the 24-hour urine samples as 16.6 and 16.3%, respectively, of the total daily dose of magnolol (Homma *et al.*, 1993a).

Homma *et al.* (1993b) also investigated the excretion profile of magnolol in healthy and asthmatic subjects following the single administration of Saiboku-To. Urine from 10 asthmatic patients and 7 healthy male volunteers was collected at 1, 3, 6, 9, and 12-hour intervals after an oral dose of 5 g of Saiboku-To (including 2.1 mg magnolol). Urine samples were analyzed *via* rapid flow fractionation and HPLC, with and without treatment with β-D-glucuronidase. The highest excretion rates were observed in both groups 1 hour following administration of Saiboku-To, though healthy subjects had greater rates of excretion than asthmatic subjects. The elimination half-life was also significantly greater ($P<0.05$) in asthmatic patients relative to healthy volunteers, at 1.40 and 0.93 hours, respectively. Magnolol continued to be excreted until 9 hours post-administration, with 95% of the total excreted amount present as the glucuronic-acid conjugated form in both healthy and asthmatic subjects. There were no significant differences between groups in the cumulative amount of urinary magnolol excreted after 9 hours, and approximately 10% of the dosed magnolol was recovered in the urine.

Urinary metabolites present after administration of 5 g Saiboku-To were investigated in 4 healthy male volunteers (Homma *et al.*, 1997). Fasting urine samples were collected at hours 1, 3, 6, 9, and 12 post-administration, and under normal dietary conditions at 6-hour intervals thereafter, up to 72 hours. Similar to the methods described for Homma *et al.* (1993b), samples were analyzed *via* rapid-flow fractionation and HPLC, with and without treatment with β-D-glucuronidase. Eight different urinary compounds were identified, of which two, 8,9-dihydroxydihydromagnolol and magnolol, came from *M. officinalis*. 8,9-Dihydroxy-dihydromagnolol and magnolol had peak rates of excretion within 1 to 3 hours post-administration, with approximate half-lives of 1 to 2 hours. Complete excretion *via* the urinary route occurred within 12 hours for magnolol, and within 24 hours for dihydroxydihydromagnolol. Large interindividual variation in the excretion rate of dihydroxydihydromagnolol was observed between subjects.

In study by Homma *et al.* (1992), analysis of the metabolic components of Saiboku-To excreted in the urine following oral administration in human volunteers was performed. Rapid-flow fractionation (RFF), followed by silica gel HPLC with a multichannel ultraviolet absorption detector was used to extract, isolate, and identify the compounds in the urine. Healthy volunteers (3 male) were administered 5 g of Saiboku-To at 9:00 am after a 12-hour fast, during which subjects were permitted to drink water at their discretion. Each 5 g dose of Saiboku-To contained 2,100 µg of magnolol and 20 µg of 8,9-dihydroxydihydromagnolol. Subjects fasted for an additional 9 hours, and drank 100 mL of water every 2 hours after administration of the test substance. Two asthmatic patients aged 44 and 66 also participated in the study under the same conditions as the healthy subjects; however, the asthmatic patients were not restricted from food, water, or medication (*i.e.*, theophylline) prior to, or after administration of the test substance. Urine samples were collected from the healthy study participants just prior to administration of Saiboku-To, and at 1, 3, 6, 9, 12, and 24 hours after administration. In asthmatic patients, urine was again collected just prior to administration of the test substance, and at 2, 4, 6, and 8 hours after dosing.

The urine samples from the healthy and asthmatic patients were pre-treated with β-D-glucuronidase from beef liver. RFF followed by HPLC was performed, and the ultraviolet (UV) spectra for the resulting chromatogram peaks were compared to standards. The peaks observed from samples collected after administration of the test substance were studied on the basis of pharmacokinetics, and any peak for which the UV-absorption intensity was observed to change was reported to result from administration of Saiboku-To. Three new peaks in the chromatogram were observed after pretreatment of the urine samples with β-D-glucuronidase, 2 of which were observed to be derived from *M. officinalis*, and were identified as magnolol and 8,9-dihydroxydihydromagnolol. The third peak was reported to be derived from *Glycyrrhiza glabra*. Excretion of these metabolic compounds derived from *M. officinalis* were observed to follow typical one-compartment pharmacokinetic models, with maximal values of excretion observed immediately following administration of the test substance, followed by a steady decline in excretion. The total amount of excreted magnolol was equal to 12% of the administered dose, while the amount of excreted 8,9-dihydroxydihydromagnolol was 295% of the administered dose, 3-fold higher than that contained in Saiboku-To, indicating that the presence of 8,9-dihydroxydihydromagnolol is due to metabolism by the liver rather than excretion in the urine as an unmetabolized aglycone present in the original extract.

XIII.b.1.3 Summary

In rats, orally administered magnolol is rapidly absorbed, and peak concentrations are achieved within 15 minutes of oral dosing, and based on observations of its poor bioavailability, magnolol is expected to undergo extensive first pass metabolism. The pharmacokinetics of magnolol and honokiol follow a two-compartment model and (i.v.) doses ranging from 2 to 10 mg/kg body weight display similar distribution and elimination rates indicating a linear pharmacokinetic profile. Species differences between rats and rabbits are observed, with rabbits displaying

lower volumes of distribution and higher clearance rates, resulting in a 3-fold lower half-life relative to rats (~15 *vs.* 50 min). Within 24 hours of oral administration in rats, the majority of magnolol is excreted primarily in the faeces (65%) with lesser amounts (11%) detected in the urine. There is no information detailing the pharmacokinetic profile of magnolol or honokiol in humans, and with little information detailing the excretion of magnolol or honokiol. Low quantities of magnolol and its metabolites are recovered in the urine suggesting that like rodents, humans primarily excrete magnolol in the faeces through excretion *via* the bile. This assumption is consistent with the large biphenol structure and high molecular weight (266 kDa) of magnolol, where evidence of significant glucuronidation would favour elimination in the bile (Klaassen, 2001).

Magnolol is extensively metabolized in the liver, with glucuronides present as the major metabolite in the plasma of rats following oral administration of 20 mg/kg body weight magnolol; bioavailability was reported by the authors to be 10% of the oral dose. Reduced and isomerised benzene-soluble metabolites also have been detected in rat urine and faeces, following oral administration of 50 mg/day magnolol, and were determined to be reduced (tetrahydromagnolol) and isomerised (isomagnolol) forms of magnolol accounting for 53.4% of total faecal radioactivity.

Urinary excretion of magnolol in humans appears to follow a unimodal profile, with 1 peak occurring 1 to 3 hours following administration. In contrast to the observations in rats, where glucuronides represented only 2.8% of the total radioactivity present in the urine following magnolol administration, glucuronic-acid conjugated urinary products appear to predominate in humans, accounting for 90 to 95% of the excreted amounts of urinary magnolol (Homma *et al*., 1993a,b); this difference may be attributed to species differences in the threshold for elimination of glucuronide conjugates in the urine *vs*. the bile as significant glucuronide conjugation is observed in rodent bile. Although roughly 90% of the urinary metabolites were glucuronidated in humans, the amounts of free, conjugated, and total magnolol present in the urine represented only 10 to 17% of the administered dose of magnolol. No information characterizing faecal or blood metabolites were available.

Following a single dose of 5 g of Saiboku-To containing 2.1 mg magnolol to healthy volunteers the majority of urinary metabolites were excreted as glucuronic acid conjugates. Following β-D-glucuronidase treatment 2 magnolol derived products were detected; these were identified as 8,9-dihydroxydihydromagnolol, and the parent compound (free magnolol). Other investigators have identified three magnolia bark extract metabolites in the urine of a healthy volunteer administered a single oral dose of magnolia bark extract (2 g; 16 mg of magnolol, 8 mg of honokiol) corresponding to magnolol, 8,9-dihydroxydihydromagnolol, and a propenoic acid derivative. These results suggest that, similar to structurally related compounds in the eugenol series, a minor route for the metabolism of magnolol is *via* the epoxide-diol pathway,

with the formation of the corresponding diol following the hydrolysis and oxidation of an epoxide intermediate (Fischer *et al.*, 1990).

While considerable metabolic data has been generated for magnolol, no animal or human studies investigating the metabolism of honokiol were identified from the available scientific literature. However, based on their similar structure, honokiol is assumed to be metabolized similarly to magnolol with the free hydroxyl groups subject to glucuronidation and elimination in the bile and urine.

XIII.b.2 Toxicology Studies of Crude Magnolia Bark Preparations

XIII.b.2.1 Acute Toxicity

The Pharmacology and Applications of Chinese *Materia Medica* contains information pertaining to the toxicity of Houpo, which is defined by the authors as the dried bark and root bark of Magnolia Officinalis (Chang and But, 1986). Magnolia bark extracts are reported to have low oral toxicity (Chang and But, 1986). There was no reported mortality 3 days following the administration of a single intragastric dose of 60 g of Houpo/kg body weight to mice (Murakami *et al*., 1933; Fan, 1975). The LD_{50} following intraperitoneal administration of a Houpo decoction in mice was 6.12 ± 0.038 g/kg body weight (Basic Medical Sciences Department, 1973), while the minimum lethal dose (MLD) in cats following *i.v*. injection was 4.25 ± 1.25 g/kg body weight (Basic Medical Sciences Department, 1973). It was also reported that while a dose (route of administration not indicated) of the decoction commonly used to produce muscle relaxation had no effect on the electrocardiogram (ECG) of experimental animals, large doses resulted in death *via* respiratory depression (Basic Medical Sciences Department, 1973). The composition of the magnolia bark extracts containing herbal remedies, and the species were not reported in the studies above. In addition, the authors also elude to the fact that the observations reported above regarding the toxicity of Houpo decoctions are likely due to presence of the water soluble alkaloid magnocurarine that is contained in the bark, which is therefore concentrated to some extent in Houpo extracts.

The acute oral toxicity of magnolia bark or Houpo, was evaluated in male ICR mice administered a single dose of magnolia bark extract (from the dry bark of *Magnolia officinalis*, obtained in a Chinese herbal drug store in Taiwan and evaluated by the National Research Institute of Chinese medicine) (Yang and Chen, 1997). Ethanolic extracts were prepared such that the final solution contained 0.5 g of raw herbal material in 1 mL solution. The maximum dosage tested was reportedly 50 g/kg body weight, estimated as dry weight of Houpo. The oral LD_{50} was found to be greater than 50 g/kg body weight, body weight indicating that the magnolia bark preparation as tested was of low oral toxicity.

Yang and Chen (1997, 1998) also conducted an acute toxicity study of 15 commonly used Chinese drugs and traditional herbal preparations, including magnolia bark (Houpo, from *Magnolia officinalis).* Reference dosages were determined using 1/10 to 1/5 of the oral LD_{50}, as

described above, as the maximal tolerable dose. Male adult Sprague-Dawley rats were administered extracts containing 0.5 g/mL dry weight of herbal raw material for 14 days. Two groups of 8 to 15 rats received either 5 or 10 g/kg body weight of magnolia bark extract orally *via* oesophageal intubation, while a third group was used as a control. During the course of the study, physical appearance, locomotor activity, and mortality were monitored, and body weight, food consumption, and water intake were measured. No significant differences in behavioural parameters, or food, water, or body weight were noted between groups. According to the authors, analysis of clinical chemistry and haematological values revealed that magnolia bark caused a decrease in alanine aminotransferase and creatinine, and an increase in blood urea nitrogen relative to the control group, but had no effect on haemoglobin concentration, total white blood cell counts, plasma total protein or serum albumin content. Urinalysis demonstrated an increase in protein in the magnolia bark extract treated groups. Gross and histological examination of heart, liver, lung, and kidney revealed no significant findings and no mortality was reported to occur during the experimental period. However, discrepancies between the text and the statistical presentation of results, the lack of discussion of significant differences between the 5 and 10 g/kg body weight groups, and the absence of reference to specific time points makes interpretation of the results presented by Yang and Chen (1997, 1998) difficult. In addition, the authors did not report whether values for specific parameters, were altered or were still within the normal physiological or historical range for rats. A summary of the available acute and short term toxicity studies described is presented below.

Table XIII.b.2.1-1 Summary of Acute and Short-Term Animal Toxicity Studies

Species/Strain/No. of Animals per Group per Sex	Study Duration	Route	Dose Levels and Test Item (mg/kg body weight/day)	Observations	Reference
Mice					
Male ICR	Single dose	Gavage & i.p.	Ethanolic extract of Magnolia bark extract	Oral LD_{50} > 50 g/kg bw i.p $L.D_{50}$ = 8.5 g/kg bw	Yang and Chen, 1997
*NS	Single dose	Gavage	Houpo 60 g/kg bw	No fatalities	Murakami *et al.*, 1933
*NS	Single dose	i.p.	Houpo deconcoction	i.p. LD_{50} = 6.12 g/kg bw	Basic Medical Sciences Department, 1973
Rats					
Sprague-Dawley Male (200-250g) N=8-15/group	14 day	Gavage	- Houpo dried powder 5 g/kg bw - Houpo aqueous suspension for higher dose 10 g/kg bw	- No effect on behaviour, food/water intake or, body weight. - ↓ ALA, and Creatine - ↑ BUN - ↑ urine protein	Yang and Chen, 1997
Rabbits					

Table XIII.b.2.1-1 Summary of Acute and Short-Term Animal Toxicity Studies					
Species/Strain/No. of Animals per Group per Sex	**Study Duration**	**Route**	**Dose Levels and Test Item (mg/kg body weight/day)**	**Observations**	**Reference**
*NS	Single dose	i.v.	n/a	No Mortality	Chang and But, 1986
Dogs					
*NS	Single dose	i.v.	Houpo 1 g/kg	No mortality	Chang and But, 1986
Cats					
*NS	Single dose	i.v.	Houpo deconcoction	Minimum Lethal Dose (MLD) = 4.25 mg/kg bw	Basic Medical Sciences Department, 1973

*NS = Not Stated

XIII.b.2.2 Genotoxicity and Mutagenicity of Magnolol

The mutagenicity and anti-mutagenicity of magnolol, has been investigated by Saito *et al.* (2006). Experiments were performed using the AMES test with and without metabolic activation using *S. typhimurium* strains TA98 and TA100. For both mutagenicity and anti-mutagenicity studies magnolol (dissolved in DMSO) was used at doses of 0.4 to 40 µg/plate for S*. typhimurium* strain TA98 and at doses of 0.2 to 20 µg/plate for *S. typhimurium* TA100. No evidence of mutagenicity or cytotoxicity was observed within these dose ranges (dose at which cytotoxicity was observed was not stated). The inhibitory effects of magnolol (using the same doses described above) against several direct (1-NP, MNNG, ENNG) and indirect (B[a]P, 2-AA, DMBA) mutagens was then assessed[7]. Magnolol had no effect on the mutagenic activity of the direct mutagens B[a]P, 2-AA, or DMBA. In contrast, magnolol dose-dependently inhibited the mutagenicity of the indirect (+S9) mutagens 1-NP, MNNG and ENNG by 54% (TA98), 47% (TA98), and 60% (TA100) respectively. In a separate experiment, magnolol also inhibited the number of His+ revertants in *S. typhimurium* TA98 induced by the heterocyclic amines IQ or Glu-P-2, in a dose-responsive manner. The authors concluded that magnolol was a strong suppressor of mutagenesis.

XIII.b.2.3 Other Studies and General Purported Biological Effects

The biological activities relevant to the claimed “therapeutic effects” and reported “clinical actions” of various magnolia bark preparations, or the components thereof, include anxiolytic and central depressant activity, muscle relaxation, vasorelaxation, thermoregulatory and antipyretic effects, and protective properties on gastrointestinal mucosal membranes, among others. Many of these effects may be mediated by the biological activity of the various

[7] 1-NP (1-nitropyrene), MNNG (methylnitronitrosoguanidine), ENNG (N-ehtyl-N-nitro-N-nitrosoguanidine), IQ (2-amino-3-methylimidazo[4,5-f]quinoline), Glu-P-2 (2-aminopyrido[1,2-a:3,4-d]imidazole, 2-aminoanthracene), B[a]P (benzo[a]pyrene), and DMBA (7,12-dimethylbenz[a]anthracene).

compounds found in extract. Studies describing various *in vitro, in vivo,* and *in situ* investigations on the activity of the extract or constituents of *M. officinalis* or *M. obovata* are summarized in Tables XIII.b.2.3-1 and XIII.b.2.3-2.

Gastrointestinal functions for magnolol and honokiol have been reported in the literature (Yamahara *et al.*, 1987; Zhang *et al.*, 2005; and Bian *et al.*, 2006). Yamahara *et al.* (1987) have shown that magnolol and honokiol extracts derived from Magnoliae Cortex, possess anti-ulcer properties in rodents. Gastric ulcers were induced using a variety of models in rodents. For the first set of experiments, ulcers were induced in male Wistar rats using chemical methods (HCl-ethanol or NaOH). Rats weighing 200 g were divided into groups of 5 to 6 animals who received either magnolol or honkiol at doses of 100 or 200 mg/kg body weight by oral administration. Following 1 hour, 1 mL of 150 mmol HCL-60% ethanol or the same quantity of 0.2 N NaOH were administered *via* oral gavage. After an additional hour the animals were killed and the stomach removed and lesions quantified. In animals treated with HCl-ethanol, Magnolol at doses of 100 or 200 mg/kg effectively reduced stomach lesions by 32 and 60% respectively. Honokiol was more effective than magnolol and reduced gastric lesions by 90% at the highest dose (200 mg/kg body weight), an effect that was comparable to the mucoprotective agent Sofalcone. Similar yet more potent effects were observed for NaOH induced lesions, and all doses of each agent almost completely inhibited the NaOH induced ulcers (96 to 100%), again an effect that was comparable to Sofalcone. Two other ulcer induction models were also performed, the pylorus ligation model and the water immersion stress induced model. The pylorus induced gastric mucosal membrane lesion model was also conducted in male Wistar rats, and following a 24-hour fasting period, the abdomen was opened in each animal while under surgical anaesthesia. The pylorus of each animal was then ligated, followed by administration of the test compound into the duodenum. Thirteen hours thereafter, the animals were sacrificed and ulcer formation quantified. For the water immersion stress induced gastric ulcer model, male ddY mice weighing approximately 20 g were used. Immediately following the oral administration of 100 or 200 mg/kg body weight of magnolol or honokiol, stress was induced in the mice by inserting the animals into a vinyl pipe and then submerging the pipe into a water bath up to the animals' neck for a duration of 5 hours. Only modest inhibition of gastric ulcers was observed with either agent in rats with pylorus ligation induced ulcers with a maximum inhibition of 40% relative to controls that was only significant in the honokiol treated animals ($P<0.05$). In the water immersion model neither agent had any effect on ulcer formation.

The potential "prokinetic" effect of magnolol and honokiol on gastrointestinal movement was studied *in vitro* using isolated gastric fundus strips from rats (200 to 300 g) and guinea pigs (200 to 300 g) of either sex as well as male mice (18 to 22 g); however the species were not identified (Zhang *et al.*, 2005). The magnolol and honokiol used in the study were extracted from *Magnolia officinalis Rehd. et Wils*. Isolated gastric fundus strips were prepared and mounted in an organ bath under a resting tension of 1 g and the muscular contraction measured

using a force transducer. Contraction of the smooth muscle of the fundus strip was then induced by adding acetylcholine or (Ach) or serotonin (5-HT). The ability of magnolol or honokiol to attenuate muscle contraction was determined by adding either agent to the water bath in conjunction with Ach or 5-HT. The results of the experiment showed that both magnolol and honokiol inhibited gastric fundus strip contraction in a non-competitive manner. In addition, both magnolol and honokiol non-competitively inhibited the contractility of guinea pig ileum segments induced by either Ach or $CaCl_2$. The *in vivo* effects of magnolol or honokiol in mice were also tested. Mice were administered magnolol or honokiol at oral gavage doses of 0, 0.5, 2.0, 20.0 mg/kg body weight. Nuclide and pigment methylene blue were used to measure the gastrointestinal movement of a nutritious semi-solid meal. The authors concluded that both magnolol and honokiol effectively reduced gastrointestinal transit times, an effect that was comparable to the serotonin inhibitory effects of cisapride.

These findings were confirmed by Bian *et al.* (2006), who also showed that magnolol dose dependently inhibited carbachol (CCh) or 5-HT induced muscle contractions in guinea pig colon muscle strips at concentrations ranging from 1 to 100 µM. The authors also used a velocity of pellet propulsion model where the whole colon was isolated from euthanized male guinea pigs (200 to 250 g, species not stated) and the time for artificial faecal pellets to transverse a 3 mm distance was measured under different test conditions while supported between pins submerged in a Krebs-bicarbonate medium. Magnolol dose-dependently reduced the velocity of pellet propulsion in the concentration range of 0.1 to 10 µM, and pellet propulsion was completely inhibited at concentrations above 30 µM.

Extracts of Cortex *Magnoliae officinalis* have long been utilized in traditional Chinese medicine for the treatment of lower gastrointestinal disorders (Chang and But, 1986). Although no substantive data exists supporting the claims that magnolol or honokiol can have therapeutic effects on the gastrointestinal system, the resulting exposure to magnolol and honokiol from the use of MBSE in gum and mints is highly limited and therefore would not be expected to produce effects on gastrointestinal function in humans. Similarly, findings of other studies reporting various biological activities of magnolia bark extracts were not considered relevant to the proposed use of MBSE in gum and mints.

Table XIII.b.2.3-1 *In Vitro* Studies of the Biological Effects of Magnolia Bark Extract or its Constituents					
Component	**Source**	**Species**	**Concentration**	**Effect**	**Reference**
In Vitro					
Honokiol	-	Human B-cell chronic lymphocytic leukaemia cells	20-80 µM	Induce caspase dependent apoptosis in leukaemia cells	Battle *et al.* (2005)
Honokiol	-	Human multiple myeloma cell lines (RPMI 8226-LRR5)	2-20 µg/mL	Induces apoptosis of cancer cells in caspase dependent and independent pathways	Ishitsuka *et al.* (2005)
Honokiol	-	Human prostate cancer (LNCaP and C4-2), bone marrow (HS27A) and bone-marrow derived endothelial cells	25 µM	Induced apoptosis. In C4-2 cells by activating caspases 3, 8 and 9	Shigemura *et al.* (2006)
Magnolol/ honokiol	*M. officinalis* *M. obovata*	Epstein-Barr Virus (EBV)	10 to 1,000 mol ratio/TPA	Inhibits EBV activation by TPA	Konoshima *et al.* (1990)
Magnolol/ honokiol/ 4,4'-diallyl-2,3'-dihydroxybiphenyl ether	*M. officinalis* *M. obovata*	Cultured human tumour cell lines: A549 (non-small cell lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF498 (central nerve system), and HCT-15 (colon)	ED_{50} of 3 to 5 µg/mL (magnolol and honokiol); 5.8 to 7.2 µg/mL (4,4'-diallyl-2,3'-dihydroxybiphenyl ether)	Antitumor, anticancer, significant cytotoxicity	Kim and Ryu (1999)
Magnolol	*M. officinalis* *M. obovata*	Human lung squamous carcinoma CH27 cells	10 to 100 µM	Inhibits proliferation and induces apoptosis of cell line	Yang *et al.* (2003)
Honokiol	*M. officinalis* *M. obovata*	Rat liver	IC_{50} of 2.3 x 10^{-7} M	Antioxidant, inhibits *in vitro* lipid peroxidation in rat liver mitochondria	Chiu *et al.* (1997)
Magnolol/ honokiol	*M. officinalis* *M. obovata*	*In vitro* study using eggs	62.5 to 250 µM	Antioxidant, inhibits *in vitro* lipid oxidation by TBARS	Ogata *et al.* (1997)
Magnolol/ honokiol/ obovatol	*M. obovata* *M. officinalis*	*Streptococcus mutans*	MIC of 6.25 µg/mL (magnolol and honokiol); 50 µg/mL (obovatol)	Antimicrobial, antibacterial activity	Ito *et al.* (1982)
Magnolol/ honokiol	*M. obovata* *M. officinalis*	*Porphyromonas gingivalis, Prevotella gingivalis, Actinobacillus actinomycetemcomitans, Capnocytophaga ginvivalis, Veillonella disper*	MIC of 20 to 160 µg/mL	Antimicrobial activity against periodontal pathogens	Chang *et al.* (1998)

Table XIII.b.2.3-1 *In Vitro* Studies of the Biological Effects of Magnolia Bark Extract or its Constituents

Component	Source	Species	Concentration	Effect	Reference
Magnolol/ honokiol	*M. officinalis*	*Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Micrococcus luteus, Bacillus subtilis*	MIC of 25 µg/mL	Antimicrobial activity against periodontal pathogens	Ho *et al.* (2001)
Magnolol/ honokiol	*M. obovata*	*Trichophyton mentagrophytes, Microsporium gypseum, Epidermophyton floccosum, Aspergillus niger, Crytpococcus neoformang, Candida albicans*	MIC of 25 to 200 µg/mL (magnolol); 25 to 50 µg/mL (honokiol)	Antifungal	Bang *et al.* (2000)
Magnolol/ honokiol	-	*Salmonella typhimurium* TA102	0.00005 to 50 µg/plate	Prevent UV-induced mutations	Fujita and Taira (1994)
Magnolol/ Dihydroxydihydromagnolol	*M. officinalis*	Human peripheral blood mononuclear cells	IC_{50} of 7.7 and 4.3 µg/mL, respectively	Inhibition of concanavalin A-induced blastogenesis	Taniguchi *et al.* (2000)
Magnolol/ Dihydroxydihydromagnolol/ Honokiol	*M. officinalis*	Rat liver homogenates	10 and 100 µmol/L IC_{50} of 1.9×10^{-4} mol/L for Magnolol; IC_{50} of 7.0×10^{-5} mol/L for Honokiol	Inhibition of 11β-Hydroxysteroid Dehydrogenase- induced steroid-dependent bronchial asthma	Homma *et al.* (1994)
Aqueous extract of *M. officinalis* bark	*M. officinalis*	Rat peritoneal mast cells	0.01 to 1 mg/mL	Inhibition tumour necrosis factor-α release	Shin *et al.* (2001)
In Situ					
Magnolol	*M. officinalis*	Rat thoracic aorta	Not reported	Vasorelaxation, stimulates EDRF and suppresses calcium influx	Teng *et al.* (1990)
Magnolol/ honokiol	*M. officinalis*	Rat gastric fundus strips Guinea pig ileum segments	10^{-5} to 10^{-3} mol/L and 10^{-6} to 10^{-4} mol/L	Inhibition of contraction stimulated by acetyle choline and 5-hydroxytryptamine	Zhang *et al.* (2005)

Abbreviations used: EBV – Epstein-Barr virus; ED_{50} – concentration required to produce an effect in 50% of the cells EDRF – endothelium-derived relaxing factor; MIC – minimum inhibitory concentration; TBARS – thiobarbituric acid-reactive substances; TPA – 12-*O*-tetradecanoylphorbol 13-acetate

Table XIII.b.2.3-1 In Vivo Studies of the Biological Effects of Magnolia Bark Extract or its Constituents

Component	Source	Species	Route of Administration	Duration	Dose (mg/kg body weight)	Effect	Reference
Honokiol	*Not stated*	Mouse			80	Inhibits tumour growth and prolongs life-span.	Chen *et al.* (2004)
Honkiol	*Magnolia grandiflora seed cones*	Mouse	Subcutaneous	Daily	100	Inhibits tumour growth	Bai *et al.* (2003)
Honkiol	*Not stated*	Mouse	Intraperitoneal	5 weeks	10	Additive cytotoxicity with radiation and taxotere, weight gain equal to that of controls	Shigemura *et al.* (2006)
Honkiol	*M. officinalis*	Rat	Not stated	Not stated	5	Excreted less urinary protein, lower glomerular cellularity and sclerosis. Alleviated glomerular monocyte chemoattractant protein-1 and intracellular adhesion molecule-1 levels	
Magnolol	*M. officinalis*	Mouse	Suplantar injection	Single dose	10 to 100	Antiinflammatory and analgesic, inhibits hind-paw oedema induced by carrageenan	Wang *et al.* (1992)
Magnolol	*M. officinalis*	Mouse	Intraperitoneal	Single dose	10 to 30	Antithrombotic and anti-platelet, inhibits prolonged tail bleeding time	Teng *et al.* (1991)
Magnolol	*M. officinalis*	Rat	Intraperitoneal	Single dose	25 to 100	Thermoregulation, increases heat loss, decreases heat production, with concomitant decrease in release of 5-HT in hypothalamus	Hsieh *et al.* (1998)
Magnolol	*M. officinalis*	Mouse	Orally	2 doses	100	Inhibition of ear swelling in a mouse ear swelling assay.	Taniguchi *et al.* (2000)
Magnolol/ honokiol	*M. officinalis* *M. obovata*	Mouse	Intraperitoneal	Single dose	63 to 250 (magnolol); 125 to 500 (honokiol)	CNS depressant, sedation, ataxia, muscle relaxation, loss of righting reflex	Watanabe *et al.* (1983)
Magnolol/ honokiol	*M. obovata*	Rat	Oral	Single dose	200	Antiulcer, inhibits gastric mucosal lesions induced by NaOH, HCl-ethanol in rats	Yamahara *et al.* (1987)
Honokiol	*M. officinalis* *M. obovata*	Mouse	Oral	Single dose	2 or 20	Anxiolytic, increases time spent in open arms in elevated plus-maze test	Kuribara *et al.* (1998)
				7 days	0.1 to 2		

Table XIII.b.2.3-1 In Vivo Studies of the Biological Effects of Magnolia Bark Extract or its Constituents

Component	Source	Species	Route of Administration	Duration	Dose (mg/kg body weight)	Effect	Reference
Honokiol	*M. officinalis* *M. obovata*	Mouse	Oral	7 days	0.19	Anxiolytic, increases time spent in open arms in elevated plus-maze test	Maruyama *et al.* (1998)
Honokiol	*M. officinalis* *M. obovata*	Mouse	Oral	7 days	0.2	Anxiolytic, increases time spent in open arms in elevated plus-maze test	Kuribara *et al.* (2000)
Honokiol	*M. officinalis* *M. obovata*	Rat	Intraperitoneal	Single dose	0.010 to 0.10	Protective effect (antioxidant activity) on rat hepatocytes following ischemia-reperfusion	Chiu *et al.* (1997)
Magnolol/ honokiol	*M. officinalis*	mouse	Orally	Single dose	0.01 to 0.4	Decreased gastric nuclide retention rate and	Zhang *et al.,* 2005
β-eudesmol	Not specified	Mouse	Intraperitoneal	Single dose	300	Antiepileptic, prevented convulsions induced by maximal electroshock	Chiou *et al.* (1996)
β-eudesmol	Not specified	Mouse	Intraperitoneal	Single dose	50 to 300	Antiepileptic, prevented convulsions induced by maximal electroshock	Chiou *et al.* (1997)
β-eudesmol	Not specified	Rat	Intraperitoneal	Single dose	10 to 100	Hypotensive, fall in blood pressure	Arora *et al.* (1967)
β-eudesmol	Not specified	Cat	Intravenous	Single dose	5 to 20	Hypotensive, fall in blood pressure	Arora *et al.* (1967)
Aqueous extract of *M. officinalis* bark	*M. officinalis*	Rats	Orally	Single dose	1 to 1000	Dose-dependent inhibition of compound 48/80 induced anaphylaxis, inhibition of local immunoglobulin E-mediated passive cutaneous anaphylactic reaction, reduction plasma histamine levels	Shin *et al.* (2001)

XIII.b.2.4 Human Studies of Magnolia Bark Derived Preparations

Recently, a prospective, two-group, parallel, double-blind, placebo-controlled clinical trial was conducted by Kalman *et al.* (2008) to study the effects of a proprietary blend of extracts of *Magnolia officinalis* and *Phellodendron amurense* (Relora® - 250 mg/capsule) on anxiety, stress and sleep in healthy premenopausal women (Kalman *et al.,* 2008). The 6-week study involving 42 healthy overweight (BMI of 31.2 ± 4.2 kg/m^2) female subjects (aged 25 to 50) were administered the test article, Relora®, containing *Magnolia officinalis and Phellodendron amurense* (1.5% honokiol and 0.1% berberine, respectively), or a placebo 1 capsule 3 times daily; thus, consuming 750 mg of Relora® or placebo, containing approximately 11.25 mg/day of honokiol and 0.75 mg/day of berberine. Subsequently, subjects were requested to self-report increase eating in response to stress using Spielberger STATE and TRAIT psychometric questionnaires. Additionally, blood samples were obtained at the beginning and end of the study for analysis of biochemical markers for safety parameters.

The results collected following the 6-week treatment period, the authors concluded that Relora®, which contains *Magnolia officinalis* and *Phellodendron amurense* (1.5% honokiol and 0.1% berberine) was effective in reducing temporary, transitory anxiety as well as self-perceived stress or anxiety. However, it was not effective in reducing long-lasting feelings of anxiety. Although the intent-to-treat population consisted of 40 subjects, only 26 subjects completed the study (16 participants in the Relora®-treatment group and 10 in the placebo group). Two in the Relora®-treated group and one in the placebo group withdrew from the study due to adverse events. One subject from the Relora®-treated group suffered heartburn, hands shaking, perilabial numbness, sexual dysfunction, and thyroid dysfunction; the second subject in the Relora®-treated group suffered fatigue and headaches. The subject in the placebo group complained of irritability, abdominal bloating, and tiredness. Despite these reported events, no serious treatment-related adverse events were noted in this study. Laboratory values did not indicate any significant test article-related side effects, as no significant differences in biochemical markers were observed between the Relora®-treated and placebo group.

Available clinical investigations of magnolia bark have focused on the use of magnolia bark extract as a component of traditional Asian remedies such as Banxia Houpo Tang (Hange-koboku-To in Japanese), Saiboku-To, Wuu-Ji-San, among others. For example, Iwasaki *et al.* (2002) reported the results of an investigation with Banxia Houpo Tang (BHT), a herbal remedy consisting of the bark of *M. officinalis,* the rhizome of *Pinellia ternate* and *Zingiber officinale,* the white part of *Poria cocos*, and the leaves of *Perilla frutescens*, in elderly stroke patients with aspiration pneumonia. Sixteen male and female elderly patients were randomly assigned to 1 of 2 groups. For a treatment period of 4 weeks, 7 subjects were administered 1.5 g of BHT 3 times daily 30 minutes prior to meals, while the other 9 subjects consumed 1.5 g of lactose on the same dosing regimen, for a total daily dose of 4.5 g BHT or placebo. The concomitant use of other medications was permitted, with the exception of angiotensin-converting enzyme inhibitors. A control group of 8 elderly volunteers was also

included. No adverse effects were reported in the study, and the authors also cited the long history of use of this remedy, and its clinically established safety (Iwasaki *et al.*, 2002). The actual composition of the herbal remedy and proportion of magnolia bark used in the preparation were not specified.

In a case-study reported by Hisanaga *et al.* (2002) Hange-koboku-tu (containing magnolia bark extract at unknown levels) was used in a 44-year-old Japanese male. The patient received 5.0 g/day of the herbal remedy over a period of 7 months the authors reported that no adverse effects were observed. Mantani *et al.* (2002) reported the results of a series of case studies investigating the effectiveness of Hange-koboki-tu and Kami-shoyo-san for the treatment of anxiety disorders in 4 female patients. Patients were between 33 to 59 years old, and each subject received 7.5 g of herbal supplementation for periods ranging from 12 weeks to as long as 2 years of daily continuous therapy. Markedly diminished anxiety was reported for each subject, and the treatment was considered successful by the authors. No information was noted by the authors with regards to safety monitoring or apparent side-effects. In addition, although it is well regarded that both herbal remedies contain significant amounts of magnolia bark extract, the constituents of the administered extracts were not detailed.

Oikawa *et al.* (2005) published a study detailing the prokinetic effects of Kampo herbal extracts on patients with functional dyspepsia. The study was conducted in 14 asymptomatic healthy volunteers (mean age = 36.0 years), and 15 patients with functional dyspepsia (mean age = 58.1 years). A freeze-dried extract of Hange-koboku-tu (HKT) containing 5 extracts was used for the study, and contained Magnoliae cortex at 20% wt/wt of total extract. A methanol extract of the herbal remedy was analyzed by 3D-HPLC indicating that magnolol, 6-shogaol, and honokiol were the principle constituents present in the mixture; lesser amounts of 6-gingerol, rosmarinic acid, magnoflorine, and magnocurarine were also observed in the 3D chromatogram. However, the exact quantities of the above constituents on a wt/wt basis relative to the total extract was not reported, and therefore approximate doses of honokiol and magnolol could not be determined. Each subject received HKT 3 times daily as 7.5 g oral supplements before meals; this corresponds to a daily exposure of ~4 g of magnolia bark extract per day. Both healthy subjects and individuals with functional dyspepsia received HKT for 2 weeks. The gastric emptying rate was monitored at baseline, and following 2 weeks of therapy. In healthy subjects, the gastric emptying rate following a 2-week washout period was also measured. A significant increase in gastric emptying rate was observed in both healthy volunteers (+59%; P=0.008) and in patients with functional dyspepsia (+34.8; P=0.015). HKT also resulted in a significant decrease in gastrointestinal symptoms (reflux, abdominal pain, indigestion, diarrhoea, and constipation) in subjects with functional dyspepsia (P=0.007). Clinical monitoring, or observations relating to side-effects and tolerability were not stated in the report.

Garrison and Chambliss (2006) conducted a study to determine the efficacy of a dietary supplement containing *Magnolia officinalis* and *Phellodendron amurense* in overweight women. Forty-two subjects were enrolled in this randomized placebo controlled study; subjects were healthy overweight (BMI = 25 to 34.9) female adults (ages 20 to 50 years) who reported overeating as a result of stressful situations and scored above the average mean for self-reported anxiety. Patients were randomized to receive three 250 mg capsules of NP 33-39 (Relora®) or placebo 3 times daily for a period of 6 weeks. The test article used in the study, Relora® was a proprietary blend of *M. officinalis* and *P amurense*; although the exact composition of Relora® was not stated in the study, information obtained from the company's patent (6,582, 735) suggests that the active ingredients in the supplement are mangolol, honokiol, and magnoflorine, with a honokiol concentration of 2% on a wt/wt basis. Blood samples were taken from each subject for laboratory analysis on day 0 and again on day 42, which included clinical chemistry and haematology analysis (exact indices monitored were not reported). Throughout the study, participants were weighed, submitted anxiety/depression information (questionnaires), and salivary cortisol levels were monitored at the beginning and at the completion of the study. Eighteen patients in the treatment group and 10 patients in the control group completed the study. A higher drop-out rate was observed for the control group as 9 control subjects were lost to follow-up *vs.* 1 in the treatment group. In total, 3 subjects dropped-out due to side-effects, 2 in the treatment group and 1 in the control group. The first participant complained of heartburn, shaking hands, perilabial numbness, sexual dysfunction, and thyroid dysfunction, effects that were considered by the study physician to be "possibly related" to the treatment. The second treatment subject complained of fatigue and headache, these effects were considered "probably not related" to the treatment. The control subject experienced irritability, abdominal bloating, and fatigue. No significant changes in metabolism profiles were obtained following analysis of laboratory samples. A significant ($p=0.04$) decrease in systolic blood (approximate 5 mmHg decrease) pressure was observed in the treatment group relative to controls (approximate 3 mmHg increase), an effect that appeared to be related to Relora® supplementation[8]. Relora® effectively prevented weight gain in the treatment group over the 6-week trial; in comparison to controls who gained an average of 1.5 kg ($p<0.01$), no increase in average weight was observed in the treatment group ($p<0.89$), an observation that was associated with decreased overall caloric intake in both groups (p=NS, between groups). The authors concluded that the treatment was well tolerated with safety results (clinical chemistry and haematology) comparable between groups, and that Relora® reduced blood pressure, and possible perceived stress, leading to maintenance of body weight throughout the study.

In summary, a number of clinical trial investigating the use of magnolia bark extract containing Asian herbal remedies were found in the literature (Hisanaga *et al.*, 2002; Iwasaki *et al.*, 2002;

[8] It should be noted that this observation of decreased blood pressure has also been reported in cats administered eudesmol, one of the principle volatile oils of Magnolia bark extracts (see Section 6.4.3).

Mantani *et al.*, 2002; Oikawa *et al.*, 2005; Garrison and Chambliss, 2006). Although many of these studies suggest that these herbal preparations are well tolerated, only one study (Garrison and Chambliss, 2006) evaluated safety based on the use of clinical and haematology endpoints (no tabulated summaries were included). This study was also the only one identified where the information pertaining to the chemical composition of the supplements used was reported. In the study by Garrison and Chambliss (2006), the dose of magnolia bark extract used was 750 mg/person/day for 42 days. The extract is reported to be standardized to 2% honokiol wt/wt and would correspond to honokiol intakes of 15 mg/person/day[9]. Based on the studies reported by Kotani *et al.* (2005) and Tsai and Chen, (1992) suggesting that magnolol and honokiol are present in magnolia bark extract in ratios of approximately 4:1, this would suggest that the corresponding intake of magnolol would be 60 mg/person. Full clinical monitoring and biochemical and haematological analysis was performed during the study and no evidence of toxicity was reported, further supporting the safety of MBSE consumption.

XIII.b.2.5 Studies Related Potential Irritation of Buccal Cavity

Chang *et al*. (1998) investigated the cytotoxicity of magnolol and honokiol in gingival fibroblasts exposed to magnolol and honokiol at concentrations of 10 and 100 µg/mL over a 24 hour incubation period. Listerine and chlorhexidine at the same concentrations were used as controls. Magnolol and honokiol exhibited similar cytotoxicity as Listerine and were significantly less cytotoxic than chlorhexidine ($p<0.01$). In gingival epithelial cells, magnolol and honokiol resulted in cytotoxicity similar to the levels observed for Listerine. At 1 µg/mL, magnolol exhibited a similar cytotoxic profile to Listerine, and were significantly less cytotoxic (approximately 2 to 4 times) than chlorhexidine. At 10 µg/mL cell viability was reduced to 1.65, 2.3, and 1.2% relative to controls for Listerine, magnolol and honokiol respectively compared to 0.33% for chlorhexidine, the effect was not significant.

The *in vitro* cytotoxicity of magnolol and honokiol over a 24 hour incubation period were on human normal fibroblasts and human keratinocytes (HaCaT cells) using the MTT assay was investigated by Park *et al*. (2004). In human normal fibroblasts both mangolol and triclosan displayed similar cytotoxicity profiles, with apparent cytotoxicity relative to controls only occurring at the highest test dose (10 µg/mL). In contrast, honokiol exhibited cytotoxicity at concentrations greater than 2 µg/mL, with cell viability reduced to less than 20% at the highest test concentration (10 µg/mL). In HaCaT cells, magnolol displayed poor cytotoxicity with the highest test concentration (10 µg/mL) decreasing the cell viability by approximately 20%. Honokiol and triclosan showed similar cytotoxicity patterns, with cytotoxicity apparent at concentrations ≥5 µg/mL, and cell viability was almost completely abolished at the highest test dose (10 µg/mL). The authors concluded that magnolol and honokiol have low cytotoxic effects.

[9] BMI given, therefore no dose by weight basis could be determined.

In order to evaluate the irritation effect of magnolol and honokiol, a human skin primary irritation test was conducted in 30 healthy Korean volunteers (Park *et al.,* 2004). Subjects were screened for existing skin conditions, and were interviewed regarding the use of any potentially irritating topical or systemic preparations during the month prior to the patch test. Honokiol and magnolol were applied to test patches (separately and in combination) in petrolatum at concentrations ranging from 5 to 100 µg/mL. The test substances were applied to the skin under occlusion for 48 hours, and study participants were not permitted to bathe, exercise, or perform any work tasks that could moisten the patches during this time. Test reactions were assessed at 30 minutes and 24 hours following patch removal according to the criteria developed by the International Contact Dermatitis Research Groups (ICDRG). Dermal exposure to magnolol and honokiol was not observed to result in skin irritation, nor were any adverse events reported by study participants.

XIII.b.2.6 Other Studies

A number of additional studies were identified in the literature reporting the use of Magnolia bark preparations or various Asian herbal remedies containing magnolia bark extract. Although they are of limited value in the safety determination of Wrigley's product as they either do not properly characterize the composition of the test article used in the study or do not contain relevant safety endpoints, they were included for completeness as seen in Table XIII.b.2.6-1 below.

XIII.b.2.6-1 Studies of the Biological Effects of Magnolia Bark Extracts or its Constituents in Humans							
Component	**Source**	**Species**	**Route of Administration**	**Duration**	**Dose (mg/kg body weight)**	**Effect**	**Reference**
Magnolol	Dietary supplement (Saiboku to) containing amongst other ingredients, magnolol	Human	Not reported	104 weeks	2.5 g Saiboku to 3 times daily (after meals); equivalent to 3.15 mg magnolol daily	Decrease in frequency of corticosteroid administration in responding bronchial asthmatics. No reduction in the frequency of corticosteroid administration among the non-responding subjects was reported. 'Responders' to Saiboku-To treatment exhibited higher free magnolol excretion rates than non-responders.	Homma *et al.*, 1993a
Extract of *M. officinalis*	Dietary supplement containing amongst other ingredients, *M. officinalis*	Human	Oral	3 times a day for 6 weeks	250 mg of supplement (amount of extract of *M. officinalis* not reported)	Well tolerated. Significant weight gain for placebo group but no weight gain for treatment group. (tested in overweight females age 20 to 50)	Garrison and Chambliss, 2006; Kalman *et al.,* 2006
Magnoliae cortex bark	Dietary supplement Hange-koboku-to which also contained *Hoelen, Perillae herba* and *Zingiberis rhizoma*	Human	Oral	10 days	60 (of supplement)	Decrease in frequency of choking episodes caused by sleep apnoea	Hisanaga *et al.,* 2002
Magnolia bark	Dietary supplement Hange-koboku-to which also contained *Hoelen, Perillae herba* and *Zingiberis rhizoma*	Human	Oral	4 weeks for patient 1, 6 months for patient 2 and 2 years for patient 3	7.5 g of supplement/day	No effect in patient 1, a 59-year-old women suffering from a panic disorder and agoraphobia. Patient 2: symptoms of agoraphobia disappeared after 12 weeks treatment, no return of symptoms 2.5 years after discontinuation of supplement. Patient 3: relief of panic disorder and agoraphobia after 2 weeks treatment. Attempted discontinuation caused return of symptoms so treatments were continued.	Mantani *et al.,* 2002

XIII.b.2.6-1 Studies of the Biological Effects of Magnolia Bark Extracts or its Constituents in Humans							
Component	**Source**	**Species**	**Route of Administration**	**Duration**	**Dose (mg/kg body weight)**	**Effect**	**Reference**
Magnoliae cortex	Supplement Hange-koboku-to which also contained *Hoelen, Perillae herba* and *Zingiberis rhizoma*	Human	Oral	2 weeks	7.5 g of supplement/day	Gastric emptying rate increased in healthy volunteers but after a 2-week washout returned to normal. Gastric emptying rate increased in functional dyspepsia patients and a decrease in scores for abdominal pain, indigestion and constipation but not reflux or diarrhoea.	Oikawa *et al.,* 2005
-	Banxia Houpo tang, which contains among other ingredients magnolia	-	Oral	4 weeks	4.5 g/day of herbal medicine	Decreased cough threshold in patients with aspiration pneumonia.	Iwasaki *et al.,* 2002
Extract of *M. officinalis*	Proprietary blend of patented extracts of the bark of *M. officinalis* (1.5% honokiol/capsule) and *Phellodendrom amurensel* (0.1% berberine/capsule)	Human	Oral	6 weeks	750 mg of Relora® per day (approximately 11.25 mg/day of extract of *M. officinalis* was consumed)	Relora® reduced self-perceived stress and anxiety as well as temporary, transitory anxiety. No treatment-related safety concerns or significant adverse events were reported.	Kalman *et al.*, 2008

XIII.c Safety of Other Phenolic and Alkaloid Constituents

Magnolia bark extract is rich in two biphenol compounds, magnolol and honokiol; however, it also contains essential oils known as α-, β-, and γ-eudesmol. Small amounts of plant alkaloids, and the presence of methyleugenol also is reported to be present in Magnolia barks. In contrast, MBSE utilizes the supercritical carbon dioxide chemical extraction method, which significantly reduces the content of the essential oils, and other contaminants due to the relatively low temperature of the process and the stability of carbon dioxide.

XIII.c.1 α-, β- and γ-Eudesmol

Alpha- and β-Eudesmol (Figure XIII.c.1-1) are bicyclic sesquiterpenoid alcohols derived from a naphthalenic skeletal system and a Eudalene framework (Arora *et al.*, 1967). β-Eudesmol has been isolated from *M. officinalis* and is one of the principal constituents of *Atractylodes lancea rhizoma* (Kobayashi *et al.*, 1990; Kimura *et al.*, 1994; Chiou *et al.*, 1997). Traditionally, *A. lancea* has been used as a component of Japanese medicinal preparations designed to alleviate muscular pain (Kimura *et al.*, 1994).

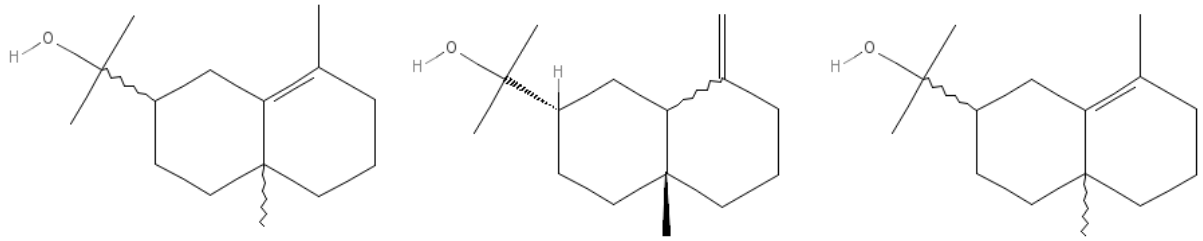


Figure XIII.c.1-1 Structures of α, β, and γ-Eudesmol (left to right respectively)

XIII.c.1.1 Estimated Daily Intake of α-, β-, and γ-Eudesmol

Proportionally, α- and β- and γ-eudesmol account for 0.7% of the content of MBSE (Table I.e-2), and appear to be present in nearly equivalent amounts (proprietary data, provided by the manufacturer). As established in Section IX.c, the 95th percentile intake of MBSE in the U.K. is estimated to be 28 mg/person/day from the use of chewing gum, and 23 mg/person/day, from the consumption of compressed mints in highest users. Therefore, the maximum daily exposure to α- and β- and γ-eudesmol from the consumption of MBSE in chewing gum and compressed mints in the U.K., respectively, would be approximately 196 and 161 µg/person/day, or 3.9 and 3.2 µg/kg body weight/day.

XIII.c.1.2 Acute Toxicity and Hypotensive Effects

Animal studies have reportedly shown that β-eudesmol displays hypotensive activity (Arora *et al.*, 1967; Harborne and Baxter, 1993). Arora *et al.* (1967) conducted a systematic investigation of the structure-activity relationship of these sesquiterpenes. To determine their effect on blood pressure, 5, 10, or 20 mg/kg body weight of either α- or β-eudesmol were administered intravenously to 12 anaesthetized cats. A fall in systolic and diastolic blood pressure of 30 to 50% was observed following the administration of 10 mg/kg body weight α- or β-eudesmol. However, the duration of action for these compounds differed. The maximum hypotensive effect was observed 15 to 20 minutes following the administration of either α- or β-eudesmol; this effect was maintained for 5 to 6 hours following the administration of β-eudesmol, while the total duration of action for α-eudesmol was only 2 to 3 hours (Arora *et al.*, 1967).

The hypotensive effect of 0, 10, 30, or 100 mg/kg body weight β-eudesmol was also investigated by Arora *et al.* (1967) following intraperitoneal injection in unanaesthetized hypertensive and anaesthetized normotensive adult albino rats. Three consecutive daily blood pressure readings were averaged to yield a value for the normal blood pressure of each rat, after which rats were divided into 4 groups of 10 or 6 rats each, for the anaesthetized and unanaesthetized experiments, respectively. In the unanaesthetized experiment, rats were administered a potassium-free diet for 30 days prior to the administration of β-eudesmol, to induce renal hypertension. Rats were injected intraperitoneally with 0, 10, 30, or 100 mg/kg body weight β-eudesmol. In both investigations, blood pressure was recorded at intervals up to 24 hours post-injection. β-Eudesmol had no effect on normotensive rats, but a dose-dependent fall in systolic blood pressure was observed in hypertensive rats within 1 hour of administering 30 or 100 mg/kg body weight β-eudesmol. Maximal hypotensive effects were observed within 2 to 3 hours, with a duration of action of 5 to 6 hours (Arora *et al.*, 1967).

As part of the same experiment, the acute toxicity of β-eudesmol was examined by administering 100, 200, 400, 800, or 1,000 mg/kg body weight β-eudesmol to 10 mice each, *via* intraperitoneal injection. Doses of 800 and 1,000 mg/kg body weight were also administered by the same route to groups of 20 mice. No mortalities were reported within the 3-day observation period. At the 2 highest doses administered, a reduction in spontaneous motor activity was observed in all mice, while ataxia was only observed in 20% of mice in these groups. No other signs of toxicity were reported (Arora *et al.*, 1967). Although the study is of limited value given that MBSE is intended to be administered orally, β-eudesmol appears to be of low toxicity in mice following intraperitoneal administration.

XIII.c.1.3 Other Reported Effects

β-Eudesmol reportedly has antiepileptic activity (Chiou *et al.*, 1996, 1997). Maximal electroshock induced convulsions and lethality in male ICR mice were prevented following the intraperitoneal injection of 300 mg/kg body weight β-eudesmol (Chiou *et al.*, 1996, 1997). A

significant decrease in tonic convulsions was observed (27%), and lethality was significantly reduced from 42 to 4%. An additive effect was also observed when sub-effective doses of β-eudesmol (50 or 100 mg/kg body weight) were administered concomitantly with 5 mg/kg body weight phenytoin (Chiou *et al.*, 1997). However, pretreatment with 300 mg/kg body weight β-eudesmol had no effect on clonic convulsions or death induced by a subcutaneous injection of 125 mg/kg body weight pentylenetetrazol or 6 mg/kg body weight picrotoxin (Chiou *et al.*, 1997). The authors suggested that this anticonvulsant activity might be due to a use-dependent blockade of sodium channels, as opposed to an effect on gamma-aminobutyric acid (GABA) or glutamate-mediated synaptic transmission (Chiou *et al.*, 1996).

β-Eudesmol has also been reported to block nicotinic cholinergic receptors (nACh) in the skeletal muscles of mice, leading to receptor desensitization (Kimura *et al.*, 1994). *In situ* investigations on the sciatic nerve-gastronemius muscles of normal and diabetic mice have demonstrated that β-eudesmol blocks the neuromuscular junction, and that these effects are greater in diabetic mice relative to normal mice (Kimura *et al.*, 1994).

The blocking effect of β-eudesmol in skeletal muscle may also be directly related to the purported antidotal properties of this compound against carbamate and organophosphate intoxication (Chang, 1998). In isolated mouse diaphragm preparations, β-eudesmol antagonized the release of acetylcholine (ACh) induced by the acetylcholinesterase inhibitors neostigmine and the organophosphorus compound diisopropyl fluorophosphate (DFP), and restored the tetanic contraction. β-Eudesmol also reduced the lethal toxicity of DFP *in vivo* following intraperitoneal administration of 300 mg/kg body weight to mice pretreated with atropine, obidoxim, or atropine and obidoxim (Chang, 1998). The authors postulated that ACh release is also readily inhibited by tubocurarine, and therefore may be mediated by an L-type calcium channel. These results suggest that β-eudesmol may have a weak curare-like action, acting *via* the nicotinic cholinergic receptor (Kimura *et al.*, 1994). However, Chang (1998) stated that no curare-like action for β-eudesmol was evident.

No studies on the absorption, distribution, metabolism, and excretion, subchronic and chronic toxicity, reproductive and developmental toxicity, carcinogenicity, mutagenicity, or genotoxicity of α- or β-eudesmol were identified from the available scientific literature. One study on the antimutagenic activity of (+)-β-eudesmol was identified (Miyazawa *et al.*, 1996). (+)-β-Eudesmol reportedly suppressed the SOS-inducing activity of the mutagens furylfuramide (2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide) and Trp-P-1 (3-amino-1,4-dimethyl-5*H*-pyrido[4,3-b]indole) in the *umu* test in *S. typhimurium* TA 1535/pSK1002, and the mutagenic activity of these compounds in the Ames test in *S. typhimurium* TA 100.

The principle secondary components of MBSE are α-, β-, and γ- eudesmol, which are found in the MBSE proposed for use in food at total concentrations ranging from 0.29 to 0.63%; this intake would correspond to maximum intakes of 196 µg/person (2.94 µg/kg body weight) eudesmol per day in the highest consumers. It has been reported that β-eudesmol can display

antihypertensive effects in SHR rats, however decreases in blood pressure required *i.v*. or intraperitoneal (*i.p*.) doses of at least 10 or 30 mg/kg body weight respectively, and no effects were observed at lower doses (Arora *et al.*, 1967). MBSE is intended for use in food, and observations of reduced blood pressure in rodents receiving high doses of eudesmol *via i.p*. or *i.v*. routes is irrelevant to the safety of MBSE under it's proposed uses. β-Eudesmol has also been reported to have curare like action in rodents (Kimura *et al.*, 1994); however discrepant findings were reported by Chang *et al.* (1998). The consumption of eudesmol from the use MBSE in mints and gums would be several thousand to a million-fold below does reported to elicit significant biological effects, and would therefore not be expected present a safety concern.

XIII.c.2 Methyl Eugenol

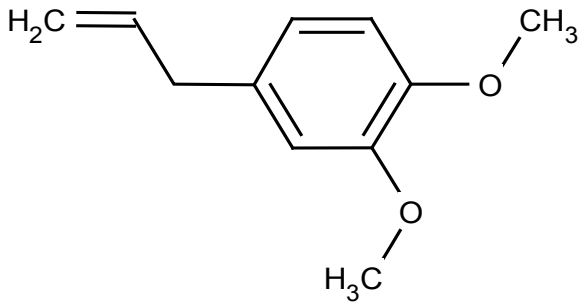


Figure XIII.c.2-1 Structure of Methyl Eugenol

Methyleugenol (Figure XIII.c.2-1) is a natural occurring constituent of a number of plants and is found in nutmeg, pimento, tarragon, basil, star anise, and fennel. The compound has also been used as a flavouring agent in a number of foods (*e.g*., jellies, baked goods, non-alcoholic beverages, chewing gum, and ice cream) at concentrations from 5 to 52 ppm and as a fragrance at concentrations from 0.002% to 0.3% (NTP, 2002). Daily per capita intake of methyleugenol has been estimated by the World Health Organization to be 73 µg per person and intakes as high as 16,900 mg per person also have been reported (WHO, 1981; Stofberg and Grundschober, 1987; National Academy of Science, 1989). Since, methyleugenol and other eugenol derivatives have been isolated from an extract of the commercially available dry powder of *M. officinalis* bark (Baek *et al.,* 1992), the William Wrigley Jr. Company has analyzed several batches of the MBSE proposed for use in food and have found trace levels of methyleugenol ranging from concentrations of 6.5 to 7.5 ppm. These levels are below the 20 ppm limit for methyleugenol, when naturally present in flavourings and food ingredients with flavouring properties for use in ready-to-eat savouries (Annex III, REGULATION (EC) No 1334/2008). Under the proposed use of MBSE in the mints and gum, 90th percentile intakes in the highest consumers (teenagers) would correspond to methyleugenol exposures of 375 ng/ person/day. Based on these intakes, which are expected to overestimate actual intakes by

several fold, it is clear that that the consumption of MBSE would not appreciably increase the intake of this compound in the diet relative to background exposure (17 µg to 18,000 µg/person) to the methyleugenol in commonly consumed foods. The small trace amount of methyleugenol in MBSE is therefore not of toxicological concern.

XIII.c.3 Alkaloids

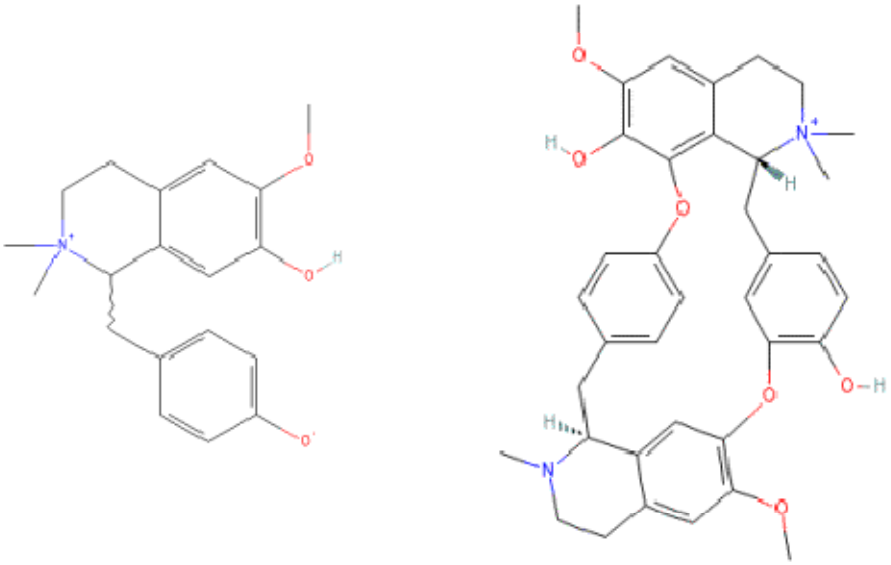


Figure XIII.c.3-1 Structure of Magnocurarine (left) and Tubocurarine (right)

Curarine alkaloids (figure XIII.c.3-1) occur in magnolia bark extracts at concentrations of up to 0.2%. Health Canada issued a warning in 2001 based on the potential tubocurarine content of 2 Chinese formulations containing magnolia bark from *M. officinalis* (Health Canada, 2001). Tubocurarine (*d-tubocurarine*) is one of the active principles of curare, which induces skeletal muscle paralysis, leading to death *via* respiratory failure. Tubocurarine is a quarternary long-acting non-depolarizing neuromuscular blocking agent that acts by competing for nicotinic cholinergic receptors, primarily at the neuromuscular junction. *d-Tubocurarine* has a long duration of action, and a high frequency of side effects, such as histamine release, ganglionic blockade, and blockade of vagal responses. Due to the presence of a positively charged quarternary ammonium group, however alkaloids such as tubocurarine have poor lipid solubility, are unable to penetrate the blood brain barrier at the dosages used clinically, and cannot act centrally, limiting their neuromuscular blocking effects to the peripheral nervous system (Wingard and Cook, 1977). Similarly, their high polarity results in poor absorption from the gastrointestinal tract following ingestion (Wingard and Cook, 1977; Martindale, 1989). In the United States, the common dosage of tubocurarine, as tubocurarine chloride, in adults is 3

mg/mL by i.v. administration, with 6 to 9 mg employed initially, followed by 3 to 4.5 mg if required, 3 to 5 minutes after initial administration of the drug, and maintenance doses of 3 mg thereafter (Martindale, 1989). Doses ranging from 300 to 500 µg/kg body weight have been used in children, while a dosage of 200 to 250 µg/kg body weight is suggested for premature infants or neonates up to 28 days of age (Bennett *et al.*, 1976; Martindale, 1989).

Information regarding the toxicity of the benzyltetrahydroisoquinoline derivative, magnocurarine, is scant, with studies limited to its identification and isolation from medicinal plant sources. However, magnocurarine is reportedly the major toxic component in magnolia bark (Chang and But, 1986). Consistent with its quarternary ammonium alkaloid structure, a curare-like action for magnocurarine, including muscle relaxant and anti-tremor effects, have been reported in frog rectus abdominus muscle and nerve-muscle preparations from albino rats, as well as *in vivo* in rabbits and chickens (Ogyu, 1954; Inoue, 1957; Chang and But, 1986). Similar to tubocurarine, the presence of a quaternary ammonium group and permanent positive charge would suggest that magnocurarine is relatively insoluble in lipids, and therefore poorly absorbed from the gastrointestinal tract. Magnocurarine reportedly has a faster onset, but shorter-lived and weaker action than tubocurarine; however, is minimally absorbed and rapidly excreted following oral administration, resulting in low blood levels *via* the oral route (Chang and But, 1986). The LD_{50} of magnocurarine in mice following intraperitoneal administration is reported to be 45.55 mg/kg (Ogyu, 1953).

The crude bark of *M. officinalis* may contain low levels of alkaloids, ranging from 0.1 to 0.2% magnocurarine (Chang and But, 1986). Based on a specification limit for total alkaloids, the maximum levels of magnocurarine that could be present would be limited to 100 ppm. Tubocurarine is not present in MBSE at the level of detection of 2 ppm. Assuming (although highly unlikely) that 100% of the alkaloid content of MBSE was magnocurarine, 90th percentile intakes of MBSE *via* compressed mints (5.9 mg) and chewing gum (28 mg) would result in the ingestion of 2.8 and 0.59 µg magnocurarine/person/day respectively. Given the very low concentrations of the curarine alkaloids which are 30 to 75 times below that used therapeutically (*i.v*), in combination with the known poor absorption of these compounds following oral administration, the presence of curarine alkaloids in MBSE is not expected to present a safety hazard following the consumption of MBSE under it's intended uses.

EVALUATION AND CONCLUSION

Approval is sought under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients, for the approval of Magnolia Bark Supercritical Carbon Dioxide Extract (MBSE) isolated from *Magnoliae officinalis* subspecies *biloba*, as a food ingredient (European Parliament and the Council of the European Union, 1997). In this regard, the William Wrigley Jr. Company intends to market compressed mints and chewing gum containing MBSE at a level of 0.2% in chewing gum and compressed mints. Under these proposed food uses the highest exposures to MBSE were estimated to occur in teenagers, where mean and 95th percentile exposures were determined to be 6.5 and 28 mg/person/day (0.13 and 0.56 mg/kg body weight) for gum consumption and 7.3 and 23.2 mg/person/day (0.15 and 0.46 mg/kg body weight) for mint consumption respectively. On a mg/kg basis, highest exposures were estimated from children, where the intake of MBSE was determined to be 0.21 and 0.60 mg/kg body weight in mean and 95th percentile gum users respectively; corresponding mint consumption in these users was 0.22 and 1.04 mg/kg body weight per person per day. The estimated exposures in users of both products were considered on an additive basis. On an absolute basis, combined exposure to MBSE from gum and mints would be approximately 14 and 50 mg/person/day for mean and 95th percentile highest users (teenagers). Highest combined exposures on a mg/kg basis would be 0.5 and 1.6 mg/kg body weight per day for mean and 95th percentile child users.

It should be noted, however, that in addition to the standard overestimate of intake that occurs with intake analysis, chewing gum release experiments conducted by Wrigley show that roughly 50% of the MBSE incorporated into gum is not released and therefore intakes would be greatly reduced relative to the estimated levels which are calculated assuming 100% release of MBSE from the gum matrix. MBSE intakes from gum use will also be exaggerated due to the fact that gum intakes were estimated based on calculations using all gum categories, and MBSE is only intended to be incorporated into sugar-free gum and mints. Finally, estimating exposure on an additive basis through summation of 95th percentile intakes also is expected to significantly overestimate MBSE consumption.

Magnolia bark has been used historically in traditional Asian remedies for thousands of years without apparent indication of safety concern. Various extracts derived from magnolia bark are also widely available in dietary supplement products at recommended doses of 200 to 800 mg magnolia bark extract/person/day. The proposed uses of MBSE as described herein, has been determined to be Generally Recognized as Safe, and several Wrigley products have been introduced to the U.S. marketplace without apparent reports of adverse effects.

The safety of the commercial MBSE preparation has been evaluated for safety *via* a standard battery of mutagenicity/genotoxicity studies, and subchronic toxicity testing in rodents; all studies were conducted under GLP and in compliance with regulatory standards for the safety

evaluation of chemicals/food ingredients. The commercial preparation used in the toxicity studies was within the specifications proposed by Wrigley's for use as a food ingredient and is of high purity. Wrigley's MBSE was non-mutagenic and non-genotoxic in bacterial reverse mutation assays and in mammalian chromosomal aberration studies conducted in the presence and absence of metabolic activation. Following administration of MBSE at doses ranging from 625 mg/kg body weight to 2,500 mg/kg body weight, MBSE was also shown to be non-genotoxic in a mouse *in vivo* micronucleus assay. The results of the rat toxicity studies, both of which were conducted in-line with current Good Laboratory Practices (cGLP) and U.S. FDA Redbook 2000 guidelines were unremarkable. NOAELs of 480 and 240 mg/kg body weight/day, the highest doses tested were determined from the 21- and 90-day studies respectively. Based on highly conservative estimates of exposure to MBSE under the proposed uses in gum and mints, the NOAEL of 240 mg/kg body weight from the subchronic study represents an intake greater than 480 and 150 times the estimated mean and 95th percentile intakes of MBSE in children, the highest estimated exposure group on a body weight basis. Wrigley has conducted several studies in healthy humans evaluating the breath freshening capacity of MBSE when added to mints and gum. No evidence of adverse effects was reported by the study investigators, and MBSE containing gum and mints are expected to be well tolerated.

Metabolism studies in rodents and humans indicate that magnolol, the principal ingredient in MBSE, is readily glucuronidated. The major difference between species is reflected in the degree to which glucuronidated metabolites are excreted in the urine *vs.* the bile, an effect that is likely due to the species difference in the M.W cut-off for excretion of glucuronidated metabolites in the bile. The primary metabolite detected in both humans and rodents is magnolol 2-O glucuronide. The metabolite 8,9-dihydroxydihydromagnolol has also been reported in a number of studies, and metabolism to a propenoic acid derivative, has been reported in one subject. Both the dihydroxy and propenoid acid derivatives are present in free and glucuronidated form. Significant de-glucuronidation by bacterial glucosidases occurs in the gut and a number of side-chain isomerised (isomagnolol) and reduced metabolites (tetrahydromagnolol) have been detected in rodent faeces; evidence of enterohepatic circulation was reported. No metabolite specific to humans was identified in the literature review. Excretion in the bile and metabolism *via* glucuronidation appears to be the preferential metabolic fate of magnolol in rodents and humans; therefore, the rodent toxicity studies are an appropriate animal for the safety assessment of MBSE in humans.

A review of additional animal studies conducted with various crude Magnolia Bark preparations were conducted for the sake of completeness. In general Magnolia bark extracts are of low toxicity and no effects relevant to the safety of MBSE under the proposed conditions of use were identified.

Magnolia bark (Houpo) has been used historically as a traditional Asian herbal remedy without apparent indication of a safety concern. A number of clinical studies have been conducted in humans using Chinese herbal preparations containing magnolia bark (*e.g.*, Houpo, Saiboku-tu) (Iwasaki *et al.*, 2002; Oikawa *et al.*, 2005; Garrison and Chambliss, 2006). Unfortunately, with the exception of the study by Garrison and Chambliss (2006), little information is available regarding the chemical composition of the supplements used in the studies and it is therefore difficult to determine exposure levels of the various neolignans and phenolic compounds found in the magnolia bark preparations that were used. However, a study conducted by Garrison and Chambliss (2006) stated that full clinical monitoring and biochemical and haematological analysis was performed with no evidence of toxicity reported in subjects consuming magnolia bark containing supplements at a dose of 750 mg/person/day (approximately 15 and 60 mg/person per day of honokiol and magnolol respectively) for 42 days. This study further supports the safety of MBSE consumption in humans at the proposed estimated intake level.

MBSE induced irritation of the oral cavity following chewing gum use was considered unlikely based on the following observations. First, exposure levels per serving are low (1.5 mg), and Wrigley's have shown that 50% of the MBSE added to each gum pellet is released within the first 6 minutes of chewing, the majority of which is presumably swallowed, and the remaining 50% is unavailable for significant oral exposure as it is retained within the gum matrix due to the high hydrophobicity of magnolol and honokiol; therefore prolonged exposure to significant levels of MBSE are not expected during regular chewing gum use. In addition, any MBSE subsequently released during prolonged chewing periods would be expected to be diluted and removed from the oral cavity due to the significant increase in saliva production that is reported to occur during chewing gum use (Dawes and Macpherson, 1992). No evidence of adverse effects attributable to oral irritation of the buccal cavity was reported following the use of MBSE (0.2%) in mints and gum during three product specific studies conducted by Wrigley. A primary irritation skin patch test conducted in healthy Korean volunteers at concentrations ranging from 5 to 100 µl, did not produce evidence of irritation. This further supports the safe use of MBSE for use in mints and gum, and reduces the probability of oral irritation.

The principle secondary components of MBSE are α-, β-, and γ- eudesmol, which are found in the MBSE proposed for use in food at total concentrations ranging from 0.29 to 0.63%; this intake would correspond to maximum intakes of 196 µg/person (2.94 µg/kg body weight) eudesmol per day in the highest consumers. It has been reported that β-eudesmol can display antihypertensive effects in SHR rats, however decreases in blood pressure required *i.v*. or intraperitoneal (*i.p*.) doses of at least 10 or 30 mg/kg body weight respectively, and no effects were observed at lower doses (Arora *et al.*, 1967). MBSE is intended for use in food, and observations of reduced blood pressure in rodents receiving high doses of eudesmol *via i.p*. or *i.v*. routes is irrelevant to the safety of MBSE under it's proposed uses. β-Eudesmol has also been reported to have curare like action in rodents (Kimura *et al.*, 1994); however discrepant findings were reported by Chang *et al.* (1998). The consumption of eudesmol from the use

MBSE in mints and gums would be several thousand to a million-fold below does reported to elicit significant biological effects, and would therefore not be expected present a safety concern.

The William Wrigley Jr. Company analyzed several batches of the MBSE proposed for use in food and found levels of methyleugenol ranging from 6.5 to 7.5 ppm. These levels are below the 20 ppm limit for methyleugenol, when naturally present in flavourings and food ingredients with flavouring properties for use in ready-to-eat savouries (Annex III, REGULATION (EC) No 1334/2008). Based on the proposed consumption of MBSE in the mints and gum, 90th percentile intakes in the highest consumers (teenagers) would correspond to methyleugenol exposures of 375 ng/person/day. Based on these intakes, it was determined that the consumption of MBSE would not appreciably increase the intake of this compound in the diet relative to background exposure (17 µg to 18,000 µg/person) to the compound in commonly consumed foods. The small trace amount of methyleugenol in MBSE is not of toxicological concern.

Magnolia bark also has been reported to contain trace amounts of 2 alkaloids, magnocurarine and tubocurarine. Both compounds display poor lipid solubility due to the presence of a positively charged quarternary ammonium group, and tubocurarine is unable to penetrate the blood brain barrier at the dosages used clinically, and therefore cannot act centrally, limiting its anaesthetic effects to the peripheral nervous system (Wingard and Cook, 1977). Both compounds are poorly absorbed and tubocurarine is therefore considered inactive when administered orally (Wingard and Cook, 1977; Martindale, 1989). Based on a specification limit for total alkaloids, the maximum levels of total curarine compounds that could be present would be 100 ppm. The 95th percentile intake of MBSE *via* chewing gum (up to 51 mg) within the United Kingdom would result in the theoretical maximum ingestion of 5.1 µg curarine alkaloids/person/day. The levels of curarine intake are approximately orders of magnitude below the levels used therapeutically (*i.v*). Given the very low concentration of curarine alkaloids that are expected be present in the extract and the fact that they are poorly absorbed, it is not expected that these constituents are of toxicological concern following consumption of MBSE under the proposed uses.

Estimates of total exposure to MBSE from addition to the proposed food uses is 14.0 and 50 mg (0.28 and 1.0 mg/kg body weight/day) in mean and 95th percentile heavy users (teenagers). Highest exposures on a body weight basis were determined for children, where consumption was estimated to be 0.5 and 1.6 mg/kg body weight per day. Based on the highly conservative methodology used in estimating exposures under the proposed uses, these estimates are considered gross overestimates of the true exposure from the proposed uses.

Following a critical review of available literature, MBSE would be considered safe and suitable for use in gum and mints under the proposed use level based on the following. The product is produced in compliance with current Good Manufacturing Practices (cGMP) using a

supercritical extraction process resulting in a product that is highly pure, and low alkaloid impurities. The results of the 90-day product specific subchronic toxicity studies using MBSE meeting product specifications conducted consistent with U.S. FDA Redbook 2000 guidelines, where no adverse observable effects were noted when incorporated into the food of male and female SD rats at levels that are 480- and 150-fold above the highest expected intakes (all-user mean and 95th percentile high teenagers) under the proposed uses; That MBSE is non-mutagenic and non-genotoxic. The metabolic profile of magnolol is very similar in rodents and humans; magnolol has been reported to be readily glucuronidated and excreted in the bile. No metabolites specific/unique to humans were reported. MBSE is to be used in mints and gum, ensuring limited exposure. Garrison and Chambliss (2006) reported that human's ingested a magnolia bark extract containing 15 mg of honokiol and ~60 mg magnolol for 42 days and there were no adverse clinical or haematological effects. The safe use of MBSE is supported by product specific clinical trials conducted at the University of Illinois in Chicago. There was no evidence of oral irritation or acute toxicity.

CONCLUSION

From a critical evaluation of the data and information summarized in this Application, it is concluded that the use of Magnolia Bark Supercritical Carbon Dioxide Extract meeting food grade specifications (as described herein) and manufactured in accordance with current Good Manufacturing Practices, is safe and suitable for the uses proposed in gum and mints.

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