**Bureau Nieuwe Voedingsmiddelen**
**Novel Foods Unit**

# Extract van magnoliabast

# Magnolia bark extract

Tweede beoordeling van de veiligheid voor de consument, volgens de Europese verordening 258/97 betreffende nieuwe voedingsmiddelen en nieuwe voedselingrediënten

Second opinion regarding consumer safety, in accordance with European Regulation 258/97 concerning novel foods and novel food ingredients

aan/to:

de Minister van Volksgezondheid, Welzijn en Sport
the Minister of Health, Welfare and Sport

Nr. 2010-04 BNV, Den Haag, 25 september 2010
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# Inhoudsopgave



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# Beoordeling

### Inleiding

Aan de orde is een tweede beoordeling volgens de Europese Verordening 258/97 (EG97, EG97a), over het gebruik als nieuw voedingsmiddel van een extract uit de bast van de boom *Magnolia officinalis.* De aanvraag is ingediend door William Wrigley Jr Company die het extract uitsluitend wil verwerken in speciale kauwgom en *mints* die zijn bedoeld om de adem fris te houden.

In het kader van de Europese toelatingsprocedure is deze tweede beoordeling uitgevoerd door het Bureau Nieuwe Voedingsmiddelen van het College ter Beoordeling van Geneesmiddelen. Het bureau heeft hiervoor de Commissie Veiligheidsbeoordeling Nieuwe Voedingsmiddelen geraadpleegd, hierna genoemd 'de commissie VNV'.

### Eerste beoordeling

De eerste beoordeling van de aanvraag voor markttoelating is verricht in het Verenigd Koninkrijk door de *Advisory Committee on Novel Foods and Processes* (ACNFP). De ACNFP vermeldt dat de aanvrager advies heeft ingewonnen van het nationaal regelgevend agenschap voor geneesmiddelen in verband met de mogelijke medicinale werking van het extract als antibacterieelmiddel. Dit Engelse agentschap meent dat het extract niet als een geneesmiddel moet worden beschouwd mits er minder dan 3 mg per portie consumentenproduct is verwerkt. De ACNFP stelt nadrukkelijk dat claims die verband houden met de antibacteriële eigenschappen, waarmee in de Verenigde Staten van Amerika dit type kauwgom en *mints* vergezeld gaan, in de EU niet zijn toegestaan omdat deze beweringen als medische claims worden gezien.

Op verzoek van de ACNFP heeft de aanvrager meerdere malen aanvullende informatie verstrekt waaronder gegevens over de samenstelling en stabiliteit van het nieuwe extract. Daarnaast heeft de aanvrager een aantal kwesties, waarover de Engelse beoordelaars in het bijzonder bezorgd waren voor de consumentenveiligheid, nader toegelicht. Een belangrijk punt voor de ACNFP is dat op basis van betrouwbare analysen de aanwezigheid van eiwit kan worden uitgesloten. Ook is de bezorgdheid over mogelijke farmacologische en toxicologische effecten weggenomen. Daarnaast heeft de aanvrager uiteengezet op welke wijze de consumentenproducten in de markt zullen worden gezet. Gebaseerd op het totaal aan informatie acht de ACNFP een risico voor de consument niet waarschijnlijk bij het voorgestelde gebruik van maximaal 0,2 % extract in kauwgom en *mints*.

### Bevindingen van de Commissie VNV

De commissie VNV heeft geen bezwaar tegen de toelating als nieuw voedingsmiddel van het extract van magnoliabast en is het eens met de positieve beoordeling door de ACNFP. Wel maakt zij daarbij een enkele kanttekening en aanvullende opmerkingen. De commissie VNV heeft haar oordeel gebaseerd op de informatie in het dossier (inclusief de aanvullende gegevens) waarvan de samenvatting is opgenomen als bijlage A, en de eerste beoordeling door de ACNFP, toegevoegd als bijlage B.

Beschrijving van het product en productieproces. Volgens de aanvrager bevat het extract twee 'actieve' bestanddelen: magnolol (>92,5 %) en honokiol (>0,5 %). Deze bifenolverbindingen vormen samen minimaal 94% van het extract. Daarnaast zijn geringe hoeveelheden alfa-, beta- en gamma-eudesmol aanwezig. Voor het totaal aan eudesmol

vermeldt de productspecificatie een maximaal gehalte van 2 %. Het vochtgehalte is 0,5 %. Aan onzuiverheden kan het extract sporen van methyleugenol (<50 µg/g) en bepaalde alkaloïden (totaal <100 µg/g) bevatten. Ook heeft de aanvrager grenswaarden in de productspecificatie opgenomen voor zware metalen (totaal en afzonderlijk gehalten voor arseen en lood). Op basis van aanvullende samenstellingsgegevens van 15 recente productiepartijen stelt de ACNFP vast dat de aanvrager een extract vervaardigt met een consistent hoge zuiverheid van minimaal 98 % aan magnolol en honokiol.

Voor het nieuwe product wordt uitsluitend de soort *Magnolia officinalis* subsp. biloba gebruikt die tot de familie van de Magnoliaceae behoort. De aanvrager verklaart dat de bast afkomstig is van gecultiveerde bomen die voldoende zijn volgroeid. De bast van de stam, takken en wortels wordt eerst schoon en dan fijn gemaakt en vervolgens geëxtraheerd met superkritisch koolzuur ($CO_2$). Het ruwe extract wordt gezuiverd door het in alcohol op te lossen en te herkristalliseren. Beiden chemicaliën zijn van farmaceutische kwaliteit. Stabiliteitsgegevens tonen aan dat het hoofdbestanddeel magnolol onveranderd aanwezig blijft in de consumentenproducten gedurende minimaal 10 maanden.

De aanvrager beheerst de microbiologische risico's voldoende en de kwaliteit lijkt gewaarborgd. Volgens de commissie VNV is het niet bekend of de bomen met pesticiden zijn behandeld en of er antischimmelmiddelen zijn gebruikt na het oogsten van de schors. Informatie over eventuele residuegehalten van dergelijke bestrijdingsmiddelen ontbreken in het dossier. Afgezien van deze kanttekening heeft de commissie geen opmerkingen en sluit zij zich aan bij de eerste beoordeling.

Geschatte inname. Het nieuwe extract zal worden toegepast in speciale soorten kauwgom en *mints* die niet bedoeld zijn als snoepgoed maar voor een frisse adem. Het formaat van deze consumentenproducten is maximaal 1,5 gram en het extractgehalte hierin zal ten hoogste 0,2 % zijn. Dus elke portie kauwgom of *mints* bevat niet meer dan 3 mg van het extract. De eerste beoordelaars concluderen dat de te verwachten inname van het extract in absolute zin het hoogste (95$^e$ percentiel) is bij tieners die dagelijks 28 mg met kauwgom en 23 mg met *mints* zullen binnenkrijgen. Deze schatting is gebaseerd op consumptiegegevens uit een database voor het Verenigd Koninkrijk en betreffen alleen gebruikers van de genoemde producttypen. Per kg lichaamsgewicht wordt de hoogste inname (95$^e$ percentiel) geschat op 0,6 mg/kg bw/d (met kauwgom) en 1,04 mg/kg bw/d (met *mints*) door kinderen in de leeftijd van 4 -11 jaar.

In aanvulling op de ACNFP noemt de commissie VNV ook de gemiddelde innamegegevens. Afhankelijk van het gebruikte producttype komt dit voor kinderen neer op zo'n 4-6 mg extract per dag, voor tieners op 6-7 mg extract per dag en voor volwassenen op 3-5 mg extract per dag. Omgerekend naar lichaamsgewicht is de gemiddelde inname bij kinderen ruwweg tweemaal meer dan die van tieners, te weten 0,3 en 0,2 mg/kg bw/dag respectievelijk met *mints* en kauwgom. Bijbehorende veiligheidsmarges van deze blootstellingen ten opzichte van de NOAEL (zie 'toxicologische informatie' hieronder) zouden neerkomen op 800 en 1200.

De commissie VNV beschikt uit andere dossiers over gegevens van kauwgominname bij de algemene bevolking. Hieruit blijkt dat de blootstelling bij een worst case scenario zou neerkomen op ruwweg 10 g kauwgom per dag, overeenkomend met 20 mg extract. Dit is ruwweg vergelijkbaar met de hoeveelheid ingeschat door de aanvrager. Daarnaast noemt

de commissie dat er bij Nederlandse jongvolwassenen op consumptiedagen uitschieters (95e percentiel) kunnen voorkomen van ongeveer 24 gram suikervrije kauwgom (RIVM03)

Het dossier bevat geen analyse van het totaal gebruik van zowel kauwgom en *mints* op één en dezelfde dag. Hoewel de aanvrager het onwaarschijnlijk acht dat consumenten grootgebruikers zullen zijn van beide type producten, kan dit volgens de commissie VNV niet worden uitgesloten. Maar ook dan bedraagt de veiligheidsmarge nog minimaal 140 en dat is volgens de commissie VNV voldoende.

Toxicologische informatie. Het dossier bevat onderzoeken waaruit blijkt dat het extract niet mutageen is voor bacteriën en dat het erfelijk materiaal van zoogdiercellen niet aantoonbaar wordt beschadigd. In het 90 dagen toxicologisch onderzoek bij ratten was de hoogste blootstelling 240 mg/kg bw/dag. De aanvrager beschouwt dit als de NOAEL (*No Observed Adverse Effect Level*) omdat hierbij geen nadelige effecten werden waargenomen. De commissie VNV merkt hierbij op dat het totaal gehalte aan magnolol en honokiol in de gebruikte teststof minimaal 90 % was. De recent geproduceerde partijen hebben zelfs een zuiverheid van minimaal 98 % aan magnolol en honokiol.

Volgens de aanvrager is na consumptie van het nieuwe product de blootstelling aan magnolol en honokiol buiten de mondholte beperkt. Echter, betrouwbare gegevens uit onderzoek met mensen over weefselspiegels en mogelijke farmacologische effecten ontbreken. De commissie VNV is het eens met de aanvrager dat, gezien de lage dosering het niet waarschijnlijk is dat er ongewenste effecten zullen optreden. Net als de ACNFP meent zij dat er geen aanwijzingen zijn dat de functie van het maagdarmkanaal nadelig wordt beïnvloed. Evenzo is de commissie VNV niet bezorgd dat het nieuwe extract, gezien de veronderstelde antibacteriële werking, schadelijk zal zijn voor de darmmicrobiota.

Volgens de ACNFP heeft de aanvrager het voldoende aannemelijk gemaakt dat de hoofdbestanddelen door omzettingen in de lever worden afgebroken waarna de gevormde metabolieten het lichaam zullen verlaten, voornamelijk met de feces. Consumptie van het nieuwe extract zal dit orgaan niet significant meer belasten, omdat de hoeveelheid magnolol plus honokiol maar een fractie vormt van het totaal aan natuurlijke bestanddelen in onze dagelijkse voeding die door de lever worden gemetaboliseerd. Het is daarom niet waarschijnlijk dat er ongewenste interacties met medicijnen zullen optreden, ook bij grootgebruikers van de nieuwe producten. De commissie VNV is het hiermee eens.

Een belangrijk aandachtspunt voor de commissie VNV zijn de schadelijke organische verbindingen in de magnoliabast, die in het extract kunnen terechtkomen. Zoals vermeldt onder "productspecificatie" hierboven, kan methyleugenol in het extract aanwezig zijn tot een maximum gehalte van 50 µg/g. Het toevoegen aan voedingsmiddelen van methyleugenol als zuivere stof stof (aroma) is verboden omdat het wordt verdacht van kankerverwekkende eigenschappen voor de mens. Door het gebruik van bepaalde kruiden als basilicum en nootmuskaat komt deze verbinding wel gewoon voor in onze dagelijks voeding. In vergelijking met recente schattingen van de hoeveelheden methyleugenol die men zo binnenkrijgt (Smi10), is de verwachte inname van deze verbinding met het extract van magnoliabast slechts marginaal. In het licht van deze achtergrondblootstelling is de commissie VNV het eens met de aanvrager dat consumptie van het nieuwe extract, zelfs door liefhebbers van de voorgestelde producten, het gezondheidsrisico niet significant vergroot.

Het totaal aan eudesmolverbindingen, die afkomstig zijn van de etherische oliën in de magnoliabast, is gespecificeerd als ten hoogste 20 mg/g. De aanwezigheid van wateroplosbare curarine alkaloïden in het extract is volgens de productspecificatie beperkt tot maximaal 100 µg/g (totaal gehalte en 2 µg/g specifiek voor tubocurarine). Volgens de commissie VNV heeft de aanvrager voldoende onderbouwd dat hiervan geen nadelige gezondheidseffecten zijn te verwachten.

Het rapport van de eerste beoordeling bevat enkele overzichten uit het dossier van uiteenlopende onderzoeken bij proefdieren en mensen. De onderzochte preparaten zijn complex samengestelde Aziatische kruidenmiddelen en de magnoliabastextracten in deze mengsels zijn afkomstig van andere firma's dan van de firma Wrigley. De aanvrager erkent dat deze extracten niet representatief zijn voor zijn product. Belangrijker nog is volgens de commissie VNV, dat door de vermenging met andere stoffen het onmogelijk is eventuele gezondheidseffecten te relateren aan één van de afzonderlijk componenten. Zij concludeert dat deze gegevens niet relevant zijn voor de huidige veiligheidsbeoordeling.

### Conclusie

De commissie VNV is het eens met de ACNFP dat de veiligheid van het extract van magnoliabast bij de voorgestelde toepassing van ten hoogste 0,2 % in kauwgom en *mints* voldoende is onderbouwd.

### Referenties

EG97 Verordening (EG) nr. 258/97 van het Europees Parlement en de Raad van 27 januari 1997 betreffende nieuwe voedingsmiddelen en nieuwe voedselingrediënten. Publicatieblad van de Europese Gemeenschappen 1997; L43: 1-6.

EG97a Aanbeveling (EG) nr. 97/618/EG van de Commissie van 29 juli 1997 betreffende de wetenschappelijke aspecten en de presentatie van de informatie die nodig is om aanvragen voor het in de handel brengen van nieuwe voedingsmiddelen en nieuwe voedselingrediënten te ondersteunen alsmede het opstellen van de verslagen van de eerste beoordeling uit hoofde van Verordening (EG) nr. 258/97 van het Europees Parlement en de Raad. Publicatieblad van de Europese Gemeenschappen 1997; L253: 1-36

RIVM03 Resultaten van de voedselconsumptiepeilingen 2003. RIVM, Bilthoven.Zie http://www.rivm.nl/vcp/gegevens/jongvolwassenen/ en http://www.rivm.nl/vcp/publicaties/jongvolwassenen/index.jsp

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# Assessment (courtesy translation)

### Introduction

This report describes a second assessment under European Regulation 258/97 (EC97, EC97a) of the use as a novel food of an extract from the bark of the tree *Magnolia officinalis.* The application for authorisation was submitted by William Wrigley Jr Company, which wishes to use the extract exclusively in special chewing gum and mints intended for freshening the breath.

The second assessment was performed by the Dutch Novel Foods Unit of the Medicines Evaluation Board, in accordance with the European authorisation procedure. The Unit consulted the Committee on the Safety Assessment of Novel Foods (referred to below as 'the VNV Committee') regarding its assessment.

### Initial assessment

The initial assessment of the application for market authorisation was conducted in the United Kingdom by the *Advisory Committee on Novel Foods and Processes* (ACNFP). The ACNFP reports that the applicant sought clarification from the UK's Medicines and Healthcare products Regulatory Agency (MHRA) regarding the possible medicinal effect of the extract, as an antibacterial agent. The MHRA advised that the use of the extract would not be medicinal, providing that less than 3 mg was present in each portion of consumer product. The ACNFP stated explicitly that claims relating to the antibacterial properties, as made regarding such chewing gum and mints in the USA, would be illegal in the EU, since they would be regarded as medical claims.

At the request of the ACNFP, the applicant provided additional information several times, including details of the extract's composition and stability. The applicant also addressed a number of consumer safety questions, regarding which the UK assessors were particularly concerned. In their view, it was important that the applicant did demonstrate the absence of protein by reliable analyses. Furthermore, the assessors' concerns regarding possible pharmacological and toxicological effects were removed. The applicant additionally explained how the consumer products would be placed on the market. Having considered all the available information, the ACNFP concluded that the novel extract for use in gums and mints at the specified use level of 0,2 % is unlikely to pose a risk to consumers.

### Findings of the VNV Committee

The VNV Committee has no objection to approval of the extract of magnolia bark as a novel food and concurs with the ACNFP's positive assessment. The VNV Committee does, however, add various comments. The VNV Committee based its assessment on the information in the dossier (together with the additional data), which is summarized in annex A, and the first assessment by the ACNFP, which is included as annex B.

Description of the product and the production process. According to the applicant, the extract contains two 'active' components: magnolol (>92.5%) and honokiol (>0.5%). These biphenol compounds together make up at least 94% of the extract. Small amounts of alpha, beta and gamma eudesmol are also present. The product specification states that the total eudesmol concentration does not exceed 2%. The moisture content is 0.5%. The impurities that the extract may contain are traces of methyleugenol (<50 µg/g) and certain alkaloids (total

<100 µg/g). The product specification also gives upper concentration limits for heavy metals (for total heavy metals and arsenic and lead separately). On the basis of additional data on the composition of fifteen recent production batches, the ACNFP concluded that the applicant produces an extract of consistently high purity, made up of at least 98% magnolol and honokiol.

The novel product is made exclusively from the species *Magnolia officinalis* subsp. biloba, which belongs to the Magnoliaceae family. The applicant states that the bark comes from sufficiently mature, cultivated trees. The bark of the stem, branches and roots is first washed and then crushed and extracted with supercritical carbon dioxide ($CO_2$). The raw extract is purified by dissolving it in alcohol and then recrystallizing it. Both chemicals are of pharmaceutical quality. Stability data indicate that the main component, magnolol, remains unchanged in the consumer products for at least ten months.

The applicant manages the microbiological risks adequately and the quality appears to be assured. According to the VNV Committee, it is not apparent whether the trees are treated with pesticides, or whether fungicides are used after harvesting the bark. The dossier contains no information regarding possible residual concentrations of such chemicals. Otherwise, the Committee has no comments and concurs with the first assessment.

Anticipated intake. The novel extract is to be used in special types of chewing gum and mints, which are not intended as confectionary but for breath freshening purposes. These consumer products will be available in portions of up to 1.5 grams, with an extract concentration of up to 0.2%. Hence, each portion of chewing gum or mints will contain no more than 3 mg of the extract. The first assessors concluded that the highest (95$^{th}$ percentile) absolute intake was likely to be in teenagers consuming 28 mg of extract with chewing gum or 23 mg of extract with mints per day. This estimate is based on consumption data for the UK, which relate only to users of the relevant product types. The highest (95$^{th}$ percentile) extract consumption per kilo bodyweight is estimated at 0.6 mg /kg bw/d (chewing gum) and 1.04 mg/kg bw/d (mints) in children between four and eleven years old.

To supplement the ACNFP's report, the VNV Committee cites the average consumption figures from the dossier. Depending on the product type used, the average for children amounts to 4-6 mg of extract per day; the figure for teenagers is 6-7 mg of extract per day and that for adults is 3-5 mg of extract per day. Converted into consumption per kilo bodyweight, those figures equate to an average for children that is about twice that for teenagers, i.e. 0.3 and 0.2 mg/kg bw/day respectively for mints and chewing gum. The safety margins between these levels of exposure and the NOAEL (see 'Toxicological information', below) are 800 and 1200.

The VNV Committee is in possession of chewing gum consumption data for the general population, as provided in other dossiers. These data indicate that, in the worst-case scenario, exposure would be about 10 g of chewing gum per day, which equates to 20 mg of extract. This is broadly similar to the applicant's estimate. In addition, the VNV Committee mentions that peak intakes (95$^{th}$ percentile) of about 24 g sugar-free chewing gum by Dutch young adults (age 19-30) have been observed on a single consumption day within the survey (RIVM03).

The dossier provides no data regarding the overall consumption of people who use both chewing gum and mints on the same day. Although the applicant considers it unlikely that anyone would be a heavy user of both products simultaneously, the VNV Committee does

not believe that this possibility can be excluded. However, even for such a user, the safety margin would be at least 140, which the VNV Committee regards as sufficient.

Toxicological information. The dossier contains study data indicating that the extract is not mutagenic for bacteria and has no observable damaging effect on genetic material from mammal cells. In a 90-day toxicological study with rats, the highest exposure studied was 240 mg/kg bw/day. The applicant regards that figure as the NOAEL (*No Observed Adverse Effect Level*), because no adverse effects were observed at this test dose. The VNV Committee notes that the overall magnolol and honokiol concentration in extract used in the toxicological study was at least 90%. Moreover, recently produced batches of the extract were found to be of even higher purity, i.e. at least 98% magnolol and honokiol.

According to the applicant, exposure to magnolol and honokiol outside the oral cavity is low following consumption of the novel product. However, no reliable tissue concentration data or pharmacological effect data from research with humans are provided. The VNV Committee concurs with the applicant that, in view of the low dosages, undesirable effects are unlikely. Like the ACNFP, the VNV Committee believes that there is nothing to suggest that there might be an adverse effect on gastrointestinal function. Furthermore, the VNV Committee, like the ACNFP, is not concerned that, because of its presumed antibacterial effect, the novel extract might be harmful to the intestinal flora.

The applicant satisfied the ACNFP that the extract's main components are metabolized in the liver and the resulting metabolites excreted, primarily in the faeces. Consumption of the novel extract is not expected to place a significant additional burden on the liver, because the combined amount of magnolol and honokiol involved is just a fraction of the total amount of natural food components that the liver is capable of metabolizing on a daily basis. Undesirable interaction with pharmaceutical products was not therefore considered likely by the ACNFP, even in heavy users of the novel products. The VNV Committee concurs.

The VNV Committee believes that the harmful organic compounds in the magnolia bark, which can end up in the extract, warrant consideration. As indicated above, under 'Product specification', methyleugenol can be present in the extract in concentrations of up to 50 µg/g. The addition of pure methyleugenol (as flavouring) to food is prohibited, because it is suspected of being carcinogenic to humans. However, this compound is present in our normal diet, since it occurs in herbs and spices, such as basil and nutmeg. Moreover, in comparison with recent estimates of the amounts of methyleugenol that people typically consume from such sources (Smi10), the anticipated intake of this compound with the novel extract is only marginal. In view of the level of background exposure, the VNV Committee agrees with the applicant that consumption of the extract of magnolia bark, even by heavy users of the proposed products, would not constitute a significant additional health risk.

The total concentration of eudesmol compounds originating from the essential oils in the magnolia bark is specified as being up to 20 mg/g. According to the product specification, the overall amount of water-soluble curarine alkaloids present in the extract does not exceed 100 µg/g, of which no more than 2 µg/g is accounted for by tubocurarine. From the information provided by the applicant, the VNV Committee is satisfied that no adverse health effects may be expected to arise from the presence of these compounds.

The report on the first assessment contains several summaries of data, provided by the applicant, relating to various studies with experimental animals and humans. The preparations investigated were Asian herbal remedies, whose composition was complex and which contained magnolia bark extracts prepared by companies other than Wrigley. The

applicant accepts that the extracts in question are not representative for the proposed products. More significantly in the VNV Committee's eyes, the fact that the investigated remedies contained magnolia bark extract in combination with other substances makes it impossible to ascribe any observed health effects to any individual component. The VNV Committee accordingly concludes that the data in question are not relevant to the current safety assessment.

### Conclusion

The VNV Committee concurs with the ACNFP that the applicant has satisfactorily demonstrated the safety of using the extract of magnolia bark as proposed, i.e. at the maximum use level of 0.2 % in chewing gum and mints.

### References

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# A Samenvatting van het dossier / Summary of the dossier

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

## SUMMARY

The William Wrigley Jr. Company
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# APPLICATION FOR THE APPROVAL OF MAGNOLIA BARK SUPERCRITICAL CARBON DIOXIDE EXTRACT (MBSE) FROM *MAGNOLIA OFFICINALIS*

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

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

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## ADMINISTRATIVE DETAILS

### Name and Contact Details for Correspondence

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## 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)*. 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.

***Summary***

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”[1]. The essential information requirements corresponding with this classification are outlined and discussed in separate sections in this summary (Recommendation 97/618/EC - Commission of the European Communities, 1997).

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.

# I SPECIFICATION OF THE NOVEL FOOD

## Chemical Identity

Chemical Name (for the two major components of MBSE): 5,5’-diallyl-2,2’dihydroxybiphenyl (Magnolol)

5,3’-diallyl-2,4’-dihydroxybiphenyl (Honokiol)

CAS Number: 528-43-8 (Magnolol)

35354-74-6 (Honokiol)

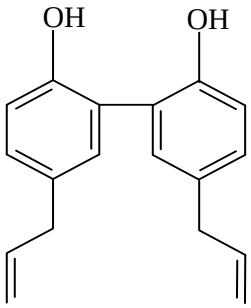
Constituents: Magnolia Bark Supercritical Carbon Dioxide Extract (MBSE) is comprised (≥94%) of the following two compounds: Magnolol and Honokiol.

Molecular Formula: $C_{18}H_{20}O_5$ (Magnolol and Honokiol)

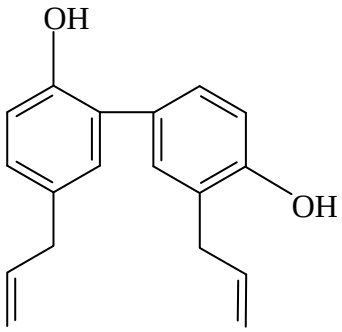
Structural Formula:

[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 releva

***Summary***




**Magnolol**




**Honokiol**

Molecular Weight: (MW) 266.34 daltons (Magnolol and Honokiol)

## Product Specification

The product specifications for MBSE are presented in Table I-1.

**Table I-1 Product Specifications for Magnolia Bark Supercritical Carbon Dioxide Extract**

| Parameter | Specification | Test Method |
|---|---|---|
| **General Specifications** | | |
| 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 |
| Heavy metals (as lead) | 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 |
| **Microbiological Specifications** | | |
| 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 |
| Moulds and yeasts | 100 cfu/g | Most Current ed. of FDA-BAM or equivalent |

## Analytical Information

Analytical data has been provided to show the absence of any potentially toxic inherent or external contaminants, specifically impurities and heavy metals. Data supporting the

absence of microbial contamination also was provided. The material is thoroughly characterized to a high purity using validated High Performance Liquid Chromatography (HPLC) methods. The product specification supports a high purity product, and is limited to a minimum of 94% honokiol and magnolol; restrictions on the percentage of eudesmol, alkaloids, methyl eugenol and lead contaminants also are included to ensure that a food grade product is consistently manufactured. Analysis of six commercial batches of MSBE confirms the product is manufactured in a consistent manner that meets the product specification. When stored and used under the appropriate conditions, or at elevated temperatures and humidity, MSBE remained virtually unchanged in compressed mints and gum.

## II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

MBSE is obtained from *Magnolia officinalis* bark using a supercritical carbon dioxide extraction method, a widely utilized food manufacturing process. The raw material is dried, sliced, crushed and extracted with $CO_2$. The supercritical $CO_2$ extract is then dissolved and re-crystallized to yield the final product. The use of a supercritical $CO_2$ production process produces a product of high purity (≥ 94% magnolol + honokiol), and ensures that the water soluble curarine alkaloids (magnocurarine), and other impurities are kept to a minimum in the final product. In addition 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.

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

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). 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).

### 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 ANTICIPATED INTAKE/EXTENT OF USE OF THE NOVEL FOOD

### Anticipated Use and Use-Levels

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, that were considered representative of Wrigley's commercial product. 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

[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

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. Based on this information the MRHA was in agreement that the proposed uses of Wrigley's MBSE would not be considered medicinal.

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

## 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).

## Estimated Consumption of MBSE from Proposed Food Uses

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, which results in the retention of magnolol and honokiol in the gum matrix, exposure to these constituents from use in chewing gum is expected to be overestimated by approximately 50%.

Based on a proposed maximum use level of MBSE in mints and gum at concentration of 0.2% of the finished product, the highest exposures to MBSE under the proposed food uses were estimated to occur in teenagers, where mean and 95$^{th}$ 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 95$^{th}$ 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 95$^{th}$ 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 95$^{th}$ percentile child users.

**Table IX.c-1 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 95$^{th}$ 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.

**Table IX.c-2 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 95$^{th}$ 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..

## X INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE

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 (U.S.), 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. 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 THE NOVEL FOOD

The proposed use of MBSE in mints and gum is to provide breath freshening characteristics to these products. As described above, 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 THE NOVEL FOOD

A summary of the toxicology information relating to Wrigley's MBSE, magnolol, honokiol, and crude magnolia bark preparations are described below.

### Animal Toxicology Studies Conducted with MBSE

The no-observed-adverse-effect level (NOAEL) for a 21-day dose-range finding toxicity study conducted on Wrigley's MBSE in male and female Sprague-Dawley rats is 480 mg/kg body weight/day, the highest dose tested (Liu *et al.*, 2007). The MBSE test article used for the experiment contained 94% magnolol and 2.0% honokiol[3]. The animals were randomly assigned to 5 groups of 5 males and 5 females and administered 0, 60, 120, 240, or 480 mg/kg body weight of MBSE extract per day incorporated into the diet.

In the 90-day study, Sprague-Dawley rats were randomly assigned to 4 groups of 20 males and 20 females and fed diets supplemented with 0, 60, 120, and 240 mg/kg body weight of MBSE per day for both males and females.[4] No mortality, ophthalmic abnormalities, treatment-related adverse clinical reactions, or statistically significant differences between treatment and control groups were observed for all haematological, bone marrow and coagulation parameters, absolute and relative organ weights and clinical or gross pathology were observed. Body weights, food consumption, clinical chemistry measurements, and

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

urinalysis did not significantly differ between the female treatment groups and the control group mostly, and any noted differences noted were not considered to be toxicologically significant as they did not occur in a dose-related manner, only occurred in one sex and values were always within the laboratory's historical range. 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.

## Genotoxicity and Mutagenicity Studies Conducted with MBSE

The *in vitro* mutagenicity studies conducted with Wrigley's MSBE are summarized in Table XIII-1 below.

**Table XIII-1 Summary of *In vitro* Mutagenicity Studies on MSBE**

| Test System | Type | Results | Concentration | Reference |
|---|---|---|---|---|
| **Prokaryotic Systems** | | | | |
| *S. typhimurium* TA98, TA100, TA1535 and TA1537 | Mut (+/- S9) | Evidence of cytotoxicty[1] | 18.75 to 5,000 µg/plate | Li *et al.,* 2007 |
| *S. typhimurium* TA98, TA100, TA1535 and TA1537 | Mut (+/- S9) | Negative | 18.75, 37.5, 75, 150, and 300 µg/plate | Li *et al.,* 2007 |
| *Escherichia coli* WP2 uvrA | Mut (+/- S9) | Evidence of cytotoxicty[1] | 18.75 to 5,000 µg/plate | Li *et al.,* 2007 |
| *Escherichia coli* WP2 uvrA | Mut (+/- S9) | Negative | 18.75, 37.5, 75, 150, and 300 µg/plate | Li *et al.,* 2007 |
| **Eukaryotic Systems** | | | | |
| CHO cells | CA (-S9) | 1 and 3 aberrant chromosome observed (2.2 and 30 µg/mL, respectively)[2] | 2.2, 7, 20, 25, and 30 µg MBSE/mL | Zhang *et al*., 2008 |
| CHO cells | CA (+S9) | 1 chromatid break and 1 chromosome break were observed (7 µg/mL)[2] | 0, 1.25, 2.5, and 7µg MBSE/mL | Zhang *et al*., 2008 |
| CHO cells | CA (-S9) | 1 isochromatid break and 1 ring chromosome were observed (0.6 µg/mL)[2] | 0, 0.6, 1.7, 5, and 15 µg MBSE/mL | Zhang *et al*., 2008 |

CA = chromosomal aberration; Mut = mutation; S9 = metabolic activation
[1]reduced rate of spontaneously occurring colonies and visible thinning of the bacterial lawn
[2] Incidences of numerical chromosome changes in all 3 experiments were comparable to those from historical negative controls for this cell line

***Summary***

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[5]. In a preliminary dose range-finding study conducted in 5 male and 5 female mice, no mortality or evidence of toxicity was observed at doses of 2,500 mg of MBSE/kg body weight suspended in 0.5% aqueous carboxymethyl cellulose by oral gavage (Li *et al.,* 2007). In the main test, 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. 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.

## Human Studies Conducted with MBSE

Sixty-two healthy subjects (21 male/41 female) participated in a two-part randomized, double-blind four-way crossover design clinical study 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. 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. 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 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

[5] 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.

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. 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.

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 was provided 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.

## Other Studies Conducted with Magnolol and Honokiol

It should be noted that the test articles used in the following 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 absorption, distribution, metabolism, and excretion studies are presented for completeness and to present an understanding of the metabolic fate of magnolol and honokiol. 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. 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.

## Toxicology and Genotoxicity Studies Conducted with Crude Magnolia Bark Preparations

A summary of acute and short-term studies conducted with magnolia bark preparations is presented in Table XIII-2.

**Table XIII-2 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<br>i.p $L.D_{50}$ = 8.5 g/kg bw | Yang and Chen, 1997 |
| *NS | Single dose | Gavage | Houpo<br>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<br>- Houpo aqueous suspension for higher dose<br>10 g/kg bw | - No effect on behaviour, food/water intake or, body weight.<br>- ↓ ALA, and Creatine<br>- ↑ BUN<br>- ↑ urine protein | Yang and Chen, 1997 |
| **Rabbits** | | | | | |
| *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

No evidence of mutagenicity or cytotoxicity was observed 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 in the AMES test with and without metabolic activation for magnolol (Saito *et al.*, 2006). Magnolol had no anti-mutagenicity effect on the mutagenic activity of the direct mutagens B[a]P, 2-AA, or DMBA at doses up to 40 µg/plate. 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.

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 are difficult to substantiate, and in many cases may be mediated by the biological activity of the various impurities found in extracts, or due to the administration of large doses of the test articles; these effects are not expected under the conditions of use of MBSE. 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-3 and XIII-4. A summary of human studies of Magnolia Bark Derived Preparations is presented in Table XIII-5.

**Table XIII-3 *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) |
| 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) |

**Table XIII-3 *In Vitro* Studies of the Biological Effects of Magnolia Bark Extract or its Constituents**

| Component | Source | Species | Concentration | Effect | Reference |
|---|---|---|---|---|---|
| Magnolol/ honokiol | *M. obovata* | *Trichophyton mentagrophytes, Microsporium gypseum, Epidermophyton floccosum, Aspergillus niger, Crytpococcus neoformang, Candida albicans* | MIC of 25 to 200 µg/mL (magnolol);<br>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<br><br>$IC_{50}$ of 1.9 x $10^{-4}$ mol/L for Magnolol;<br>$IC_{50}$ of 7.0 x $10^{-5}$ 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-$\alpha$ 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<br>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-4 *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-4** | ***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-5 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 |
| 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 |

| XIII-5 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** |
| - | 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 |

## Safety of Other Phenolic and Alkaloid Constituents

Magnolia bark extract 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.

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. *Alpha*- and β-Eudesmol are bicyclic sesquiterpenoid alcohols derived from a naphthalenic skeletal system and a Eudalene framework (Arora *et al.*, 1967). 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). 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. 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.

Methyleugenol 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, 90$^{th}$ 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.

Curarine alkaloids 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, 90$^{th}$ 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 95$^{th}$ 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 95$^{th}$ 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 95$^{th}$ percentile highest users (teenagers). Highest combined exposures on an mg/kg basis would be 0.5 and 1.6 mg/kg body weight per day for mean and 95$^{th}$ 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 95$^{th}$ 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 95$^{th}$ 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.

***Summary***

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, 90$^{th}$ 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 95$^{th}$ percentile intake of MBSE *via* chewing gum (up to 51 mg) within the United Kingdom (UK) 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.

***Summary***

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 95$^{th}$ 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 95$^{th}$ 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.

From a critical evaluation of the data and information evaluated by Wrigley, it is concluded that the use of Magnolia Bark Super Critical 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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# B Eerste beoordeling / Initial assessment

**ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES**

**OPINION ON AN APPLICATION UNDER THE NOVEL FOODS REGULATION FOR EXTRACT OF MAGNOLIA BARK**

| | |
|---|---|
| **Applicant:** | **William Wrigley Jr. Company** |
| **Responsible Person:** | **Marion Balz** |
| **EC Classification:** | **2.2** |

## Introduction

1. An application was submitted to the Food Standards Agency in September 2009 by William Wrigley Jr. Company for the authorisation of magnolia bark extract as a novel ingredient in the EU. A copy of the application was placed on the Agency's website for public consultation.

2. Magnolia bark extract is obtained from the bark of the plant *Magnolia officinalis*. This plant is native to the mountains and valleys of China and, according to the applicant, has been used for centuries as part of traditional Asian remedies. Magnolia bark supercritical carbon dioxide extract (MBSE) is mainly composed of two phenolic compounds, magnolol and honokiol. The applicant intends to incorporate MBSE into two confectionery products (chewing gum and mint confectionery products) at a maximum use level of 0.2% for breath freshening purposes.

3. The application for authorisation of magnolia bark extract was prepared pursuant to Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients. Magnolia bark extract has been classified as a complex novel food from non-GM source, the source of the novel food has no history of food use in the EU (class 2.2).

### I. Specification of the novel food

Information on this aspect is provided on p. 4-8 of the application dossier

4. The applicant states that MBSE contains two major 'active' components which comprise at least 94% of the product. The primary component is magnolol (5,5'-diallyl-2,2'dihydroxybipenyl) and the extract also contains smaller amounts of honokiol (5,3'-diallyl-2,4'-dihydroxybiphenyl). MBSE is a light brownish powder, soluble in alcohol and insoluble in water. The

specification for MBSE can be found in the table below.

| **Parameter** | **Specification** |
|---|---|
| Appearance | Light Brownish Powder |
| Magnolol | 92.5% min |
| Honokiol | 0.5% min |
| Magnolol + Honokiol | 94% min |
| Total Eudesmol | 2% max |
| Moisture | 0.5% |
| **Impurities** | |
| Arsenic (ppm) | 0.5 max |
| Lead (ppm) | 0.5 max |
| Total Heavy Metals (ppm) | 10 max |
| Methyleugenol (ppm) | 50 max |
| Turbocurarine (ppm) | 2 max |
| Total Alkaloid (ppm) | 100 max |

***Discussion**: Members noted that compositional data from analyses of multiple batches of MBSE did not total 100% (range 95.7 -100.6%) and requested clarification of the identity of the remaining components. Members also requested confirmation of the absence of protein in MBSE and reassurance that quality control procedures are sufficiently robust to ensure product consistency. The applicant provided additional batch analyses data for fifteen lots of MBSE and stated that most of these batches are characterised to a consistently high purity of between 98 and 100%. The applicant also stated that individual batch analysis indicates that the majority of the product is accounted for by magnolol, honokiol and moisture content. At the Committee's request , the applicant analysed a sample of MBSE in duplicate for protein using three different methods (SDS-PAGE with Coomassie blue R250®, SDS-PAGE with silver staining and LC-MS/MS). The applicant stated that no detectable levels of protein were found in the MBSE analysed using any of the above methods. The Committee reviewed the raw data from these analyses and was reassured that protein is effectively absent from the novel ingredient.*

## II. Effect of the production process applied to the novel food

Information on this aspect is provided on p 9-12 of the application dossier

5. MBSE is obtained from the bark of *Magnolia officinalis* L, which is washed and oven dried to reduce moisture content before being crushed and extracted with supercritical carbon dioxide. The extract is dissolved in medical-grade ethanol and re-crystallised yielding MBSE.

6. MBSE is produced in accordance with Good Manufacturing Practice. The applicant states that a Hazard Analysis and Critical Control Point (HACCP) program has been implemented for the manufacture of MBSE.

7. The applicant carried out stability analyses of MBSE in chewing gum and mints over a 12 week period under accelerated storage conditions and concluded that the results demonstrate the stability of MBSE in chewing gum and mints, with minimal loss over the 12 week test period. At the request of the Committee, the applicant also provided real-time stability data for MBSE-containing gum (different flavours) over a ten month period. Magnolol content was assessed as a measure of stability and was shown to be stable within each flavour and there was no detectable degradation over 10 months of shelf-life.

***Discussion**: The Committee was satisfied with this section of the dossier and the additional data provided by the applicant.*

## III. History of the organism used as a source of the novel food

Information on this aspect is provided on p.13-15 of the application dossier

8. MBSE is obtained solely from the bark of *Magnolia officinalis* subsp. biloba. It is a species of Magnolia native to the mountains and valleys of China at altitudes of 300-1500m and it belongs to the family *Magnoliaceae*.

9. The applicant states that traditional herbal remedies containing magnolia bark, such as Banxia Houpo Tang, Saiboku-To, Hsiao-Cheng-Chi-Tang and Wuu-Ji-San, have been used for centuries as part of Asian remedies. The applicant also also states that various magnolia bark derived products are available, and these would all be regarded as traditional medicinal products. In view of this, the applicant sought clarification from the Medicines and Healthcare products Regulatory Agency (MHRA) on the medicinal status of MBSE and its proposed use in confectionery. The MHRA concluded that use of MBSE in chewing gum would not be medicinal, providing that it was limited to claims regarding breath freshening, and that the amount of MBSE did not exceed 3mg per stick. This limit is based on the potential medicinal function of the extract as an antibacterial agent and is not a safety limit.

***Discussion**: The Committee was generally content with this section of the dossier but requested an explanation for the rationale of incorporating 3 mg of MBSE into mint/gums. The applicant stated that a published study by Greenburg et al., 2007 reported that MBSE at a concentration of 0.2% displayed breath freshening properties and a 0.2% incorporation level was employed on this basis. Based on this use level and a maximum gum/mint size of 1.5 g each, each gum or mint serving would contain 3 mg of MBSE. The applicant also explains that there is also a technical limit on the use of MBSE in gum/mints because MBSE imparts unacceptable flavour characteristics to the product which are difficult to mask at incorporation levels above 0.2%. The Committee was satisfied with the applicant's response.*

## IX. Anticipated intake/extent of use of the novel food

Information on this aspect is provided on p 16-24 of the application dossier

10. The applicant intends to incorporate MBSE into gum and mints at a maximum level of 0.2%. Based on a maximum gum and mint size of 1.5g, each serving would contain up to 3mg of MBSE.

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

Summary of the individual proposed food-uses and use levels for MBSE in the UK

11. The applicant has indicated that MBSE will be added solely to mint and chewing gum products which are marketed for breath freshening purposes. MBSE will not therefore be added to bubble-gum type products or to other mint based confectionery such as 'Everton Mints'

12. The applicant has provided intake data from a range of population groups using information from the NDNS surveys which are available to the general public. On an absolute basis highest exposure to MBSE was observed in teenagers with 95$^{th}$ percentile estimates of 28 and 23 mg/person per day for gum and mints, respectively. On a mg/kg basis, exposure to MBSE in the diet was highest in children (age 4-11) at 0.6 and 1.04 mg/kg body weight per day for gum and mints, respectively.

***Discussion**: The Committee was satisfied with this section of the dossier.*

## X. Information from previous human exposure

Information on this aspect is provided on p.25 of the application dossier

13. The applicant does not view the limited use of magnolia bark products as traditional remedies to be indicative of widespread exposure to the principal components of MBSE. The applicant reports that MBSE has GRAS (Generally Recognised as Safe) status in the United States**.** MBSE-containing gum and mints have been marketed in US since June 2008 and Oct 2008, respectively. Post market monitoring for adverse reactions in the USA (2008-2009) indicated that there was one adverse report for every 11 million units sold.

14. As MBSE-containing gum and mints have been marketed in the US**,** the Committee requested details on the way in which these products are marketed. The applicant has provided details from Wrigley's US website to illustrate the way in which MBSE products are marketed in the US. While for

the EU application the applicant intends to limit claims to breath freshening properties, this appears not to be the case in the US where claims relating to antibacterial properties of the gums and mints are being made. Such claims would be illegal under EU legislation, as they would be regarded as medicinal.

***Discussion**: The Committee was satisfied with the applicant's response and did not raise any further questions/concerns on this aspect of the application.*

## XI. Nutritional information on the novel food

Information on this aspect is provided on p.26 of the application dossier

15. The addition of MBSE to mints and gum is solely for the purposes of breath freshening and exposure to the novel ingredient is not expected to have a nutritional impact on the diet.

***Discussion**: The Committee did not raise any concerns or questions on this aspect of the application.*

## XII. Microbiological information on the novel food

Information on this aspect is provided on p.7-8 of the application dossier

The applicant has provided microbiological analyses data for four different lots of MBSE which were shown to be demonstrably free from microbial contamination (*Clostridium*, coliforms, *Salmonella*, Staphylococci, mould and yeast).

***Discussion**: The Committee did not raise any concerns or questions on this aspect of the application.*

## XIII. Toxicological information on the novel food

Information on this aspect is provided on p. 27-66 of the application dossier

Subchronic toxicity

16. The applicant conducted a 21 day toxicity study on MBSE in male and female Sprague-Dawley rats (Liu *et* al, 2007). This study was primarily a pilot dose-ranging study for a subsequent 90 day study. Animals consumed MBSE in the diet at doses of 0, 60, 120, 240 or 480 mg/kg body weight per day. Although differences in certain haematological parameters were observed, the applicant notes that these were of a low magnitude and were not dose responsive or consistent between sexes, and concludes that they are therefore not of biological relevance. Serum urea nitrogen and urine sodium values were significantly higher in the 120 mg/kg body weight/day females and males, respectively. Absolute and relative thyroid weights and relative kidney weights were slightly but significantly increased in females of the high

dose group. Relative spleen weight was slightly but significantly increased in males of the 60 mg/kg bodyweight/day group. The applicant states that organ weights were within the historical range of control weights and were not accompanied by clinical, gross or pathological effects, and therefore were not toxicologically relevant. The applicant states no treatment-related side effects were observed during this study. A NOAEL of 480 mg/kg body weight was determined (the highest dose administered).

17. The applicant also provides details of a 90 day study in which male and female Sprague Dawley rats consumed MBSE in the diet at doses of 0, 60, 120 or 240 mg/kg body weight per day. Although some differences in body weight, body weight gain and food consumption were observed, the applicant states that these effects were not dose related or toxicologically significant. Differences in certain haematological parameters (total bilirubin and sodium) were observed and urinalysis revealed significantly lower potassium levels in female animals dosed at 60 mg/kg body weight per day. The applicant states these differences were not dose dependent, not observed in both sexes and not biologically relevant. The applicant concludes that a NOAEL of 240mg/kg body weight was established.

Mutagenicity and genotoxicity

18. Ames tests conducted with and without metabolic activation were negative and MBSE was non-genotoxic in Chinese hamster ovary cells with and without metabolic activation. The applicant indicates that MBSE is non-genotoxic *in vivo* as no evidence of micronucleus induction was observed in Swiss Albino (CD-1) mice receiving MBSE doses up to 2,500 mg/kg body weight. The applicant considers that these studies indicate that MBSE is not mutagenic or genotoxic.

Human studies

19. The applicant has provided details of two double-blind human studies conducted to investigate the efficacy of MBSE. The results obtained from these studies indicated that consumption of MBSE-containing peppermint mints or gum was effective in reducing oral malodour. The applicant and the study investigator stated that the MBSE-containing products were well tolerated and that use/consumption of MBSE-containing mints did not result in any adverse effects in any of the study participants in either study. Headache was reported by one of the sixty two subjects in one of the studies, which the investigators judged was possibly related to the test product.

Toxicity studies and other studies conducted with magnolol, honokiol and crude magnolia bark preparations

20. Crude magnolia bark preparations have long been used as a component of traditional Asian remedies and the majority of published studies on the properties of magnolia bark have used the crude powdered bark or extracts produced using various solvent extraction processes. The applicant acknowledges that the test articles used in these studies are not representative of MBSE, and states that the available literature on these materials has been reviewed for completeness. This review includes a reference to mortality in animals fed 'large doses' of Houpo, a decoction (water extract) of magnolia bark that is produced for muscle relaxing purposes. The applicant notes that although the composition of this decoction is poorly defined, the findings are likely to be due to the presence of a water extracted alkaloid magnocurarine, which may have been present at concentrated levels in the extract.

21. Available data from acute and short-term animal toxicity studies carried out using these magnolia bark preparations are summarised below:

| 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<br>i.p $L.D_{50}$ = 8.5 g/kg bw | Yang and Chen, 1997 |
| *NS | Single dose | Gavage | Houpo<br>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<br>- Houpo aqueous suspension for higher dose 10 g/kg bw | - No effect on behaviour, food/water intake or, body weight.<br>- ↓ ALA, and Creatine<br>- ↑ BUN<br>- ↑ urine protein | Yang and Chen, 1997 |
| **Rabbits** | | | | | |
| *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

The applicant acknowledges that various magnolia bark preparations or components thereof are reported in the literature as having claimed therapeutic effects and reported clinical actions including: anxiolytic and central depressant activity, muscle relaxation, vasorelaxation, thermoregulatory and antipyretic effects and protective properties on gastrointestinal mucosal membranes. The applicant also describes studies showing that magnolol and honokiol (the principal components of MBSE) may have beneficial effects on gastrointestinal function. The application dossier suggests that exposure to magnolol and honokiol resulting from the use of MBSE-containing gum and mints is limited and therefore effects on gastrointestinal function in humans are not expected.

In order to assess the validity of this conclusion, the Committee asked the applicant to provide data comparing levels of these compounds in the GI tract in the published studies and following exposure to MBSE from confectionery. The applicant reported that the observations described in the dossier were obtained from an uncontrolled study (Oikawa *et al*., 2005) on a herbal concoction containing many ingredients, one of which was a crude magnolia bark preparation.

As such the applicant advised that there is no credible clinical evidence to support any pharmacological effects of magnolol and honokiol on the GI tract. The applicant's response also highlighted that no GI effects were seen in the 90 day toxicity study where rats were administered MBSE in the diet at doses around 500 times higher that the estimated intake for frequent MBSE product users. The applicant's response also highlighted that post-market monitoring data also supported the lack of any pharmacological activity of MBSE.

The applicant also stated that the other studies mentioned above are for completeness and are not considered relevant to the proposed use of MBSE in gums and mints.

The Committee was satisfied that the applicant's response addressed its concerns on this point.

22. The applicant has also detailed a number of clinical trials investigating the use of Asian herbal remedies containing magnolia bark preparations that are not necessarily representative of MBSE. The applicant states that these studies suggest that the herbal preparations are well tolerated, although only one of these studies (Garrison and Chambliss, 2006) evaluated safety using clinical and haematology endpoints. In the study by Kelman *et al.,* 2008, one of forty two subjects reported side-effects which included heartburn, hands shaking and thyroid dysfunction. However, the applicant considers that these effects were not significant test-article-related effects. Similar side effects were also reported for one of forty two subjects in the study of Garrison and Chambliss, 2006, although these authors concluded that the treatment was well tolerated. These studies are summarised below and detailed in the dossier (p50-60).

| 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 was continued. | Mantani *et al.*, 2002. |

| 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 amurense* (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 |

Safety of other phenolic and alkaloid constituents

23. In addition to the two biphenol compounds, magnolol and honokiol, magnolia bark provides essential oils containing alpha, beta and gamma-eudesmol. Magnolia barks contain small amounts of plant alkaloids (magnocurarine and tubocurarine) and methyleugenol. MBSE is produced using supercritical carbon dioxide chemical extraction so that the content of essential oils and contaminants is significantly reduced.

24. The applicant states that although beta-eudesmol has been reported to display antihypertensive effects in rats, such effects required intravenous or intraperitoneal doses of at least 10 or 30 mg/kg body weight respectively and no effects were observed at lower doses. The applicant's view is that, as MBSE is intended for food use, these observations are not relevant to the current evaluation. The applicant remarks that beta-eudesmol has also been

reported to have curare like action in rodents but these findings were not consistent in the literature. The specification for MBSE limits the total eudesmol content to 2% and the applicant highlights that MBSE intake from mints and gum would be several thousand to a million fold lower than doses reported to elicit significant biological effects and would therefore not be a safety concern.

25. The applicant states that several batches of MBSE were analysed for levels of methyleugenol, noting that a 20 ppm limit of this compound that has recently been set in EU flavourings legislation for its presence ready to eat savoury products[1]. The applicant estimates that, based on the proposed consumption of MBSE in gum and mints, 90$^{th}$ percentile intakes in the highest consumers (teenagers) would result in daily exposures of 375 ng/person and would not appreciably increase the dietary intake of this compound relative to background exposure from food (17 micrograms to 18,000 micrograms/person).

26. Given the very low concentration of curine alkaloids magnocurarine and tubocurarine that are expected to be present in the extract (the specifications limit alkaloids to a maximum of 100 ppm) and the fact that these compounds are poorly absorbed, the applicant concludes that these compounds will not be of toxicological concern as a result of consuming MBSE in mints and gum.

***Discussion**: The Committee sought an explanation for the gender-specific statistically significant increases in total blood bilirubin levels (TBBL) observed during the 90 day rodent feeding study. Noting that these increases were apparently not accompanied by other signs of liver toxicity, the Committee requested a copy of the original study report in order to be satisfied about this finding. The Committee reviewed this report and was satisfied that the 90 day report contained all relevant data and that the observed increases in TBBL were not dose-related. The Committee concluded that TBBL levels in the treatment group were significantly higher because TBBL levels in the control group were aberrantly low rather than as a result of any treatment-related effect.*

*The Committee also requested further information on the metabolism of magnolol in the liver and reassurance as to whether there may be a risk of interaction with other pharmaceutical products metabolised in the liver.*

*The applicant states that the principal constituents of MBSE, magnolol and honokiol, are primarily metabolised by the liver in rodents via conjugation with*

[1] The flavourings legislation defines limits for a range of food types to which flavourings containing methyleugenol might be added. This list does not include chewing gum or other confectionery and "ready to eat savoury products" is probably the closest surrogate for comparison.

*glucuronic acid and the main elimination route is excretion in the bile. The applicant also states that there is limited information on the metabolism of magnolol and honokiol in humans, but based on available evidence glucuronidation appears to be the main metabolic route. The applicant states that a complete absorption, distribution, metabolism, excretion (ADME) profile of magnolol in humans is not available and neither are detailed metabolic data for honokiol (although given the structural similarity to magnolol the compound is expected to be metabolised similarly via conjugation of the free hydroxyl group with glucuronic acid and subsequently excreted in the bile).*

*The maximum level of MBSE consumption is a fraction of the exposure to other natural dietary components that undergo similar metabolic conjugation processes e.g. polyphenols which are found in chocolate, red wine, coffee, tea and many fruits and vegetables. The applicant considers that potential adverse drug interactions with MBSE and pharmaceuticals will be extremely unlikely. The Committee was satisfied with the information provided by the applicant relating to magnolol metabolism and potential interaction with pharmaceutical products.*

## XIV. Allergenicity and labelling

Information on this aspect is provided on p.25 of the application dossier

27. The applicant has indicated that the product will be labelled as appropriate and in accordance with EU legislation relating to the labelling presentation and advertising of foodstuffs. Claims will be limited to its breath freshening capability and that products containing MBSE will not have any medicinal or associated health or nutrition claims.

28. The applicant states that as MBSE is isolated using supercritical carbon dioxide extraction, it does not contain protein and therefore allergy concerns are not warranted.

***Discussion**: As noted above, the applicant provided data from additional protein analyses to support their statement that MBSE does not contain measurable amounts of protein and as such it is unlikely to pose a concern with respect to allergenicity. The Committee was satisfied with the applicant's response to this point.*

## CONCLUSION

The Committee has reviewed the dossier and the additional information it requested from the applicant on a number of areas:

- Improved protein analyses
- Clarification of MBSE compositional data

- Gender-specific increases in total blood bilirubin levels observed during the 90 day rodent feeding study.
- Information on the metabolism of magnolol in the liver
- Information on the shelf-life of MBSE
- Details on how MBSE products are marketed in the US
- Further information on MBSE use levels.
- Information on ecology relating to the bark stripping process of magnolia trees.

The Committee was satisfied with the information provided by the applicant in addressing all its questions or concerns and was satisfied that MBSE for use in gums and mints at the specified use level of 0.2% is unlikely to pose a risk for consumers.

**July 2010**