ITEM A

Petition for Non-organic agricultural substances allowed in or on processed products labeled as “organic” (§205.605 (b)) to be include on the National List.

ITEM B

1. **Substance name**
   Chemical name: Sulfuric acid – Food Grade (30-50%)
   Other Names: Hydrogen sulfate
                Oil of Vitriol
                Oleum
   Composition: H₂SO₄

2. **Manufacturer’s name and contact information**
   Tas-Isle Trading Pty Ltd
   41 Sunderland Street
   Moonah Tasmania 7009
   AUSTRALIA
   Ph: +61 3 6272 5288
   Fax: + 61 3 6273 1993
   Email: sales@tasisle.com.au

3. **Intended use of the substance**
   Seaweed extraction – allowance of sulfuric acid for pH adjustment
   The current petition is for allowing the use of sulfuric acid as a pH adjustment of extraction water for the production of seaweed extracts, specifically fucoidans. Fucoidans are natural polysaccharides that contain sugar monomers. The sulfuric acid does not impact on the seaweed extract, rather it is used solely as a processing aid. Liquid formations would be overtaken with bacterial growth if the pH were not lowered to levels described herein.
   No residual sulfuric acid remains in the product. The seaweed extracts are sold as ingredients into the food supplement, function food and beverage, and cosmetic markets.
   Sulfuric acid is listed in the Japan Agricultural Standard for Organic Production as well as the Australian Quarantine and Inspection Service as a pH adjustment agent in extraction water for producing sugar. Marinova has received allowance for the use of sulfuric acid as a pH adjustment agent from the Australian Quarantine and Inspection Service on the basis that the “National Standard Sub Committee is of the opinion that fucoidan is a sugar based and therefore would already be covered in the National Standard under Annex C” (Appendix A).
   Aquatic plant extract is a listed synthetic substance allowed for use in organic crop production but the extraction process is limited to the use of potassium hydroxide or sodium hydroxide solvent used is limited to that amount necessary for extraction.
   Because of this, the use of such type of acid is not commercially viable as well as the increased costs associated with its removal from the product in the purification stages of the process.

MARINOVA
249 Kennedy Drive - Cambridge
TASMANIA, AUSTRALIA 7170
Sulfuric acid, in addition to phosphoric and citric acids, is allowed as a processing aid for pH adjustment (not below 3.5) in organically processed liquid fish products for use in crop production (NOP205.601(jj)(7)). A petition has been made for sulfuric acid to be used in a similar way with livestock manures as a processing aid in the production of dehydrated manure for use in organic crop production (pH not below 5). A petition for the use for aqua plant extracts to be allowed as plant or soil amendments under 205.601(jj)(1) was made by Arcadian Seaplants where the extracts “can be pH adjusted with sulfuric, citric or phosphoric acid”.

4. Handling activities for which the substance will be used

The petition is for sulfuric acid to be used to adjust the pH of the extraction water for the production of seaweed extracts.

5. The source of the substance and a detailed description of its manufacturing or processing procedures from the basic components to the final product

The lead-chamber process, developed in 1746, was the original method for producing sulfuric acid on an industrial scale. This was superseded by the Contact Process, patented in 1831, which has now become the primary means of sulfuric acid production worldwide.

The Contact Process can be divided into three stages:

a. Preparation and purification of SO₂ — Sulfur dioxide is prepared by burning sulfur. This commonly occurs in various industrial processes such as the burning of sulfur containing fuels (coal and oil) and the smelting of various metal ores and ore concentrates. Sulfur dioxide is captured in industrial scrubbers to prevent emissions that would otherwise contribute to acid rain. The captured sulfur dioxide is further purified, concentrated prior to the catalytic conversion to sulfur trioxide (SO₃).

b. Catalytic oxidation (using vanadium pentoxide catalyst) of sulfur dioxide (SO₂) to sulfur trioxide (SO₃) — This step involves the reaction of sulfur dioxide with oxygen (O₂) at 450 - 500° C in the presence of a vanadium catalyst. The catalyst does not contribute anything chemically to the process, rather it promotes the reaction at manageable conditions.

Conversion of sulfuric trioxide to sulfuric acid — SO₃ is not dissolved in water directly to yield sulfuric acid because of the large amount of heat that is generated from the reaction that would form clouds of corrosive vapour. Instead, the SO₃ is absorbed into already formed sulfuric acid to form pyropsulfuric acid (H₂S₂O₇). This solution is then diluted with water to obtain sulfuric acid at the desired concentration.

6. Summary of any available previous reviews by State or private certification programs or other organizations of the petitioned substance.

USDA/NOP: Sulfuric acid, citric acid and phosphoric acid are currently on the National List as synthetic substances allowed for use in organic crop production under section 205.601(jj)(7) as additives to liquid fish products. The sulfuric acid is used to pH adjust the liquid fish products, preventing further degradation or creating
odorous gases. The amount of added acid shall not exceed the minimum needed to lower the pH of the product to 3.5.

**JAS**: Sulfuric acid is listed in the Japan Agricultural Standard for Organic Production where it is allowed for use in adjusting pH of the extracted water in producing sugar (i.e., a pH adjustment agent) (JAS for Organic Processed Foods, Notification No. 1606).

**AQIS**: Sulfuric acid is listed in the Australian National Standard for Organic and Biodynamic Produce where it is allowed for use in the pH adjustment of extraction water in sugar production (National Standard for Organic and Biodynamic Produce, Edition 3.4). Marinova has received approval for the use of sulfuric acid as a pH adjustment agent in the manufacture of Organic Certified Fucoïdans (Appendix 1).

**CGSB**: The Canadian General Standards Board permits the use of fish emulsions to amend and improve soil fertility. Liquid fish products can be pH-adjusted using sulfuric acid, but the amount of acid used cannot exceed the minimum amount needed to lower the pH to 3.5 (CGSB-32.311-2006, Organic Production Systems Permitted Substances Lists).

7. **Information regarding EPA, FDA and State Regulatory authority registrations including registration numbers**

According to the Australian National Occupational Health and Safety Commission (NOHSC), sulfuric acid is deemed a hazardous substance for concentrations above 5% w/w. It is known to be corrosive and can cause severe burns. Labelling requirements include “Keep locked up and out of reach of children”, “in case of contact with eyes, rinse immediately with plenty of water and seek medical advice”, and “never add water to this product”. This information is sourced from the European Union’s Annex I of the EEC Council Directive 67/548/EEC (as updated by EEC Council Directive 2001/59/EC). The concentration at which sulfuric acid is used in this petition (<1% w/w) is not considered hazardous according to NOHSC.

In 1975 the Select Committee on GRAS Substances (Report 33, CFR Section 184.1095) concluded that “There is no evidence in the available information on sulfuric acid, and on ammonium, calcium, potassium and sodium sulfates that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or that might reasonably be expected in future”.

There is no information that exposure to sulfuric acid by itself is carcinogenic. The EPA and US DHHS have not classified sulfuric acid for carcinogenic effects. IARC has not classified pure sulfuric acid for its carcinogenic effects.

The Agency for Toxic Substance and Disease Registry (US Department of Health and Human Services) has collated the following international, national and state regulation regarding sulfuric acid in air, water and other media (ATSDR 1998).
### International, National and State Regulations regarding Sulfuric acid

<table>
<thead>
<tr>
<th>Agency</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>International</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IARC</td>
<td>Carcinogenic classification: Group 1</td>
<td>IARC 1992</td>
</tr>
<tr>
<td><strong>National</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OSHA (air)</td>
<td>PEL TWA (8hr): 1mg/m³</td>
<td>OSHA 1998 (29 CFR 1910.1000)</td>
</tr>
<tr>
<td>EPA (Water)</td>
<td>Hazardous substance under the Clean Federal Water Pollution Control Act Sec 311(b)(2)(A)</td>
<td>EPA 1998a (40 CFR 116.4)</td>
</tr>
<tr>
<td>EPA (Food)</td>
<td>Exempt from tolerance for pesticide chemicals in or on raw agricultural commodities</td>
<td>EPA 1998b (40 CFR 180.1001)</td>
</tr>
<tr>
<td></td>
<td>Exempt from the requirement of a tolerance when used in accordance with good agricultural practice as a herbicide in the production of garlic and onions and as a potato vine desiccant in the production of potatoes</td>
<td>EPA 1998c (40 CFR 180.1019)</td>
</tr>
<tr>
<td>Consumer Product Safety Commission (CPSC)</td>
<td>Sulfuric acid and any consumer product containing free or chemically unneutralised sulfuric acid in a concentration of 10% or more must bear the word “poison” on its container</td>
<td>EPA 1998g (16 CFR 1500.129)</td>
</tr>
<tr>
<td>DOT</td>
<td>Forbidden for transport on passenger carrying aircraft or railcars.</td>
<td>DOT 1998 (49 CFR 172.101)</td>
</tr>
<tr>
<td></td>
<td>Domestic transportation labels: corrosive</td>
<td>DOT 1998 (49 CFR 172.101)</td>
</tr>
<tr>
<td>EPA</td>
<td>CERCLA reportable quantity 1000 pounds</td>
<td>EPA 1998d (40 CFR 302.4)</td>
</tr>
<tr>
<td></td>
<td>Extremely hazardous substance TPQ: 1000 pounds</td>
<td>EPA 1998a</td>
</tr>
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</table>

### State (Acceptable ambient air concentrations (µg/m³))

<table>
<thead>
<tr>
<th>State</th>
<th>Concentration</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arizona</td>
<td>22.5 (1 hr), 7.5 (24 hr)</td>
<td>NATICH 1996</td>
</tr>
<tr>
<td>Connecticut</td>
<td>20 (8hr), 100 (30 min)</td>
<td>CT DEP 1998</td>
</tr>
<tr>
<td>Idaho</td>
<td>50 (24 hr)</td>
<td>ID DHW 1998</td>
</tr>
<tr>
<td>Kansas</td>
<td>2.38 (annual)</td>
<td>NATICH 1996</td>
</tr>
<tr>
<td>Louisiana</td>
<td>23.8 (8 hr)</td>
<td>LO DEQ 1998</td>
</tr>
<tr>
<td>Maine</td>
<td>300 (15 min), 17 (24 hr)</td>
<td>NATICH 1996</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>2.72 (24 hr), 2.72 (annual)</td>
<td>NATICH 1996</td>
</tr>
<tr>
<td>Nevada</td>
<td>24 (8 hr)</td>
<td>NATICH 1996</td>
</tr>
<tr>
<td>State</td>
<td>Concentration</td>
<td>Reference</td>
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<tr>
<td>------------------</td>
<td>---------------</td>
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</tr>
<tr>
<td>North Carolina</td>
<td>100 (1 hr), 12 (24 hr)</td>
<td>NC DEHNR 1998</td>
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<tr>
<td>North Dakota</td>
<td>30 (1 hr), 10 (8 hr)</td>
<td>NATICH 1996</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>100 (24 hr)</td>
<td>NATICH 1996</td>
</tr>
<tr>
<td>South Carolina</td>
<td>10 (24 hr)</td>
<td>SC DHEC 1998</td>
</tr>
<tr>
<td>Vermont</td>
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<td>NATICH 1996</td>
</tr>
<tr>
<td>Virginia</td>
<td>17 (24 hr)</td>
<td>NATICH 1996</td>
</tr>
<tr>
<td>Washington</td>
<td>3.3 (24 hr)</td>
<td>WA DE 1998</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>24 (24 hr)</td>
<td>NATICH 1996</td>
</tr>
</tbody>
</table>

8. The Chemical Abstract Service (CAS) number or other product numbers of the substance and labels of products that contain the petitioned substance – Sulfuric acid
CAS number: 7664-93-9
UN numbers: 1830, 1831, 2796
Labels: Corrosive (C), Causes burns (R35) (Conc≥15%); Irritant (Xi), Irritating to eyes and skin (R36/38)(5%≤conc≤15%)
“Keep locked up and out of reach of children”(S1/2), “in case of contact with eyes, rinse immediately with plenty of water and seek medical advice” (S26), “never add water to this product” (S30), “in case of accident or if you feel unwell, seek medical advice immediately” (S45).

9. The substance’s physical properties and chemical mode of action including:
   a. Chemical interactions with other substances, especially substances in organic production

b. Toxicity and environmental persistence of sulfuric acid
Sulfuric acid is not persistent and dissipation and the buffering capacity of the soil and water are likely to return the pH to acceptable levels within a relatively short period.
Sulfuric acid is fast-acting and is quickly neutralized in the environment by reacting with the vegetation canopy (which intercepts much of the chemical), soil and water in the soil. This means that the hazards associated with this chemical rapidly decrease from the time of application.
Sulfuric acid is known to break down relatively quickly, reducing the possibility of problems with long term effects on the environment.

MARINOV A
c. Environmental impacts from its use and/or manufacture
Sulfuric acid is a pollution control byproduct of the metal smelting industry. If not turned into a product, sulfuric acid would ultimately form acid rain in the atmosphere. Consumer use (industrial, agricultural, commercial) of sulfuric acid benefits the environment by allowing the metal industry to proceed a high quality product instead of a large volume waste. If sulfuric acid is used as outlined in this petition, its environmental impact is minimalised. Only mild conditions are used during the process thus preventing the formation of sulfuric acid mist or vapours that can escape into the atmosphere. The subsequent neutralization of the acid as part of the process prevents the addition of high acid concentrations being released into the waterways and greater ecosystem.

d. Effects on human health
Sulfuric acid, like any acids, can be harmful to humans if it comes into contact with the skin or eyes and if it is swallowed or inhaled. This is detailed in the attached material safety data sheet (MSDS). There are no human dietary concerns from the use of sulfuric acid as a pesticide on potato vines. For this use, sulfuric acid was granted an exemption from tolerance requirements because it “is rapidly degraded in the environment to sulfate salts, which are of no toxicological concern and are GRAS by the FDA”
When used in the purpose of this petition, workers are exposed to low concentrations of sulfuric acid which are deemed non-hazardous (<1%w/w). All workers use appropriate personal protective equipment.
Higher concentrations are handled by delivery personnel with appropriate licenses for handling dangerous goods.
The seaweed extracts manufactured using sulfuric acid as a processing aid are used for human consumption. There is no residual sulfuric acid in the product as this has been neutralized to sulfate salts prior to isolation and purification of the extracts. The applicant has demonstrated that seaweed extracts prepared using sulfuric acid as a pH adjusting agent have not had adverse health reactions, in contrast, the extracts have exhibited health benefits (Appendices 3 – 5).

e. Effects on soil organisms, crops or livestock
The application of sulfuric acid may result in charring of the soil and reduction of the pH in soil and water (if there is contamination of adjoining surface water by spray drift). The effect of an application on soil pH is not that large; it leads to a reduction of about 0.2. Sulfuric acid is known to break down
As mentioned above, sulfuric acid is accepted for use as a pesticide on potato vines due to its ability to rapidly degrade to sulfate salts.

10. Safety information about the substance including a Material Safety Data Sheet and a comprehensive substance report from the National Institute of Environmental Health (NIEH) Studies
A Material Safety Data Sheet (MSDS) from the supplier compliant with Australian regulations regarding the format of an MSDS is attached to this petition.

MARINOVA
A comprehensive substance report from the National Institute of Environmental Health (NIEH) for Sulfuric acid (50%) could not be found.

11. Research information about the petitioned substance which includes comprehensive substance research reviews and research bibliographies, including reviews and bibliographies which present contrasting positions to those presented by the petitioner in supporting the substance’s inclusion on the National list.


ATSDR (US Dept Health and Human Services, Agency for Toxic Substances and Disease Registry) 1998. Toxicological Profile for Sulfuric Trioxide and Sulfuric Acid. (and references therein)


CGSB (Canadian General Standards Board) -32.311-2006 Amended December 2009
http://www.tpsgc-pwgsc.gc.ca/cgbs/on_the_net/organic/index-e.html

HSE (Health and Safety Executive, UK) 2009. Use of Sulfuric Acid on Potatoes
http://www.pesticides.gov.uk/farmers_growers.asp?id=62

Japan MAFF (Ministry of Agriculture, Forestry and Fisheries)

12. Petition Justification Statement

Sulfuric acid was shown to be the most effective acid to produce high quality organic fucoidan. Marinova has developed a proprietary method, known as the Maritech® process, that is specific for the extraction of fucoidan. In our trial process, we tried the use of other strong mineral acids like citric and hydrochloric acid, but they are not suited to the innovative process developed by Marinova. The only other non-synthetic acidifying materials are alginic, citric and lactic acids. These materials are ineffective, impractical, costly and limited in their availability. The use of sulfuric acid is more effective, a more practical means to provide acidity and results in a cleaner, natural and high purity product.

The use of sulfuric acid is approved as a processing aid in the extraction of sugar by the Japan Agriculture Standard and Australian Quarantine and Inspection Service (AQIS), which oversee organic certification in Japan and Australia, respectively. Because fucoidan is also sugar-based molecule, AQIS ruled that that Marinova’s activities meet this restriction of use. Sulfuric is also currently on the National List as synthetic substances allowed for use in organic crop production under section 205.601(jj)(7) as additives to liquid fish products.

Finally, human clinical trials have shown that there was no change in kidney and liver function or other key blood chemistry indicators after the ingestion of Marinova’s fucoidan
extracts. In addition, trial participants reported no adverse effects were experienced. These results indicate that the final product is safe and the use of sulfuric acid as a processing aid does not adversely affect the product.

1. Confidential business information statement
Marinova wishes to claim CBI for the intended use of this substance as described in sections 3, 4, 9a and 12 and Appendix 6. This includes all information relating to the Maritech® process. The Maritech® process by which Marinova manufactures seaweed extracts (fucoidan) (Appendix 6) is a proprietary method that has been developed in-house by Marinova over a period of several years. The method used to prepare these extracts is unique in the market place.
APPENDIX 1
Letter from AQIS National Standard Sub Committee

National Standard Sub-Committee (NSSC)

11 August 2008

Mr Mike Spivey
Marinova Pty Ltd
GPO Box 1791
HOBART TAS 7001

Dear Mr Spivey,

RE: Applications to Alter the National Standard 03/08- Sulphuric acid and 04/08 – Sodium hydroxide

The above applications submitted to the National Standard Sub Committee (NSSC) were addressed at the meeting held on 11th July 2008.

The committee discussed the information provided in the submitted applications. The committee made reference to the Annex of the National Standard where these processing aids are listed as approved for use in sugar extraction processes which led the committee to seek clarification on whether your product can be described as a sugar based product.

From the information provided, the NSSC is of the opinion that Fucoidan is a sugar based product and therefore would already be covered in the National Standard under Annex C.

The NSSC await your response. Upon review of your response, the recommendation of the NSSC will be forwarded to the Organic Industry Expert Consultative Committee (OIECC) out of session in order to cause no further delay in the outcome of your application.

Please be advised that the draft Australian Organic Standard has been released for public comment by Standards Australia and can be viewed at www.standards.com.au.

As this document will replace the National Standard when it is implemented, you may wish to address this issue in the Australian Organic Standard whilst the public comment option is available.

If you have any further questions relating to this matter, please do not hesitate to contact the NSSC Secretariat on 02 6272 3928.

Thank you

Frances Porter
NSSC Chairperson

MARINNOVA
APPENDIX 2
Material Safety Data Sheet – Sulfuric Acid
MATERIAL SAFETY DATA SHEET

1. IDENTIFICATION OF THE MATERIAL AND SUPPLIER

PRODUCT NAME: SULPHURIC ACID 50%
OTHER NAMES: SULPHURIC ACID 50%, SULFURIC ACID 140%, SULPHURIC ACID 1400%

SUPPLIER NAME: TAS-ISLE TRADING PTY LTD (ABN 68 009 485 765)
ADDRESS: 41 SUNDERLAND STREET, MOONAH, TAS, 7009
TELEPHONE: +61 3 62731993
FAX: +61 3 62731993

POISONS INFORMATION CENTRE: 131126

2. HAZARDS IDENTIFICATION

HAZARD CLASSIFICATION:
- Classified as hazardous according to the criteria of NOHSC.
- Classified as a dangerous good UN 2789 according to the criteria of ADG Code (see section 14).
- Classified as schedule 6 according to the criteria of SUSDP (see section 15).

HAZARD CATEGORY: C - Corrosive

RISK PHRASES:
- R35 - Causes severe burns.
- R41 - Risk of serious damage to eyes.

SAFETY PHRASES:
- S1/2 - Keep locked up and out of the reach of children.
- S24/25 - Avoid contact with eyes and skin.
- S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- S30 - Never add water to this product (always add product to water).
- S33/36/37/39 - Wear suitable protective clothing, gloves and eye/face protection.
- S45 - In case of accident or if you feel unwell, seek medical advice immediately (show the label wherever possible).

The information contained in this MSDS is specific to the product when handled and used neat. This product when diluted may not require the same control measures as the neat product. Check with your technical representation if in doubt.

3. COMPOSITION / INFORMATION ON INGREDIENTS

<table>
<thead>
<tr>
<th>INGREDIENT</th>
<th>CAS No.</th>
<th>PROPORTION (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfuric Acid</td>
<td>7664-93-0</td>
<td>50%</td>
</tr>
</tbody>
</table>

The ingredients below are considered either hazardous, dangerous goods or poison scheduled according to the criteria of NOHSC, ADG Code and SUSDP (respectively) at the levels used in the product.

Water 50%

Product Name: SULPHURIC ACID 50%  Page: 1 of 5
**MATERIAL SAFETY DATA SHEET**

4. FIRST AID MEASURES

**INGESTION:** For advice, contact a Poisons Information Centre (Phone Australia 131126, New Zealand 0800 794 798) or a doctor. If swallowed, do NOT induce vomiting.

**EYE CONTACT:** If in eyes, hold eyelids apart and flush the eye continuously with running water. Continue flushing until advised to stop by the Poisons Information Centre or a doctor, or for at least 15 minutes.

**SKIN CONTACT:** If skin or hair contact occurs, remove contaminated clothing and flush skin and hair with running water. Continue flushing until advised to stop by the Poisons Information Centre or a doctor.

**INHALATION:** Remove from source of exposure to fresh air. Seek medical assistance if the effects persist.

**SHOW THIS SAFETY DATA SHEET TO A DOCTOR**

**FIRST AID FACILITIES:** Potable water should be available to rinse eyes or skin. Provide eye baths and safety showers.

**NOTES TO PHYSICIAN:** Treat symptomatically. Can cause corneal burns.

5. FIRE FIGHTING METHODS

**SUITSABLE EXTINGUISHING MEDIA:** Water spray, foam, carbon dioxide or dry chemical powder.

**HAZARDS FROM CombUSTION:** The product is non-combustible but will support combustion of other materials and may emit toxic fumes including those of sulphuric acid fumes and sulphur dioxide.

**PRECAUTIONS FOR FIRE FIGHTERS AND SPECIAL PROTECTIVE EQUIPMENT:** Fire fighters should wear self-contained breathing apparatus and acid-resistant chemical splash suit to minimise risk of exposure.

**HAZCHEM CODE:** 2R

6. ACCIDENTAL RELEASE MEASURES

**EMERGENCY PROCEDURES:** Spillages are slippery. Ensure adequate ventilation, work up wind or increase ventilation. Keep spectators away - rope off the area. Avoid accidents, clean up immediately. Wear protective equipment to prevent skin and eye contamination and inhalation of mists.

**METHODS AND MATERIALS FOR CONTAINMENT AND CLEAN UP:** Contain the spill and prevent run off into confined areas, drains and waterways.

**HAZARDS WITH DRY MATERIAL:** Absorb with dry earth, sand or other non-combustible material. Neutralise with lime or soda ash. Use clean non-sparking tools to collect and seal in properly labelled drums for disposal in an area approved by local authority by laws. Wash area down with excess water to remove residual material.

**INCISTRUCTIONS FOR DISPOSAL OF CONTAMINATED MATERIALS:** Incineration of disposed material is not recommended, as it is unlikely to adequately burn.

7. HANDLING AND STORAGE

**PRECAUTIONS FOR SAFE HANDLING:** Keep containers closed at all times - check regularly for leaks or spills. Transport and store upright. Addition to water releases heat which can result in violent boiling and splattering. Always add slowly and in small amounts. Never add water to acids - always add acids to water.

Avoid eye contact and repeated or prolonged skin contact and breathing in mists. Do not eat, drink or smoke in contaminated areas. Always remove contaminated clothing and wash hands before eating, drinking, smoking or using the toilet. Wash contaminated clothing and other protective equipment before storage or re-use.

**CONDITIONS FOR SAFE STORAGE:** Store in the original container, in a cool, dry, well-ventilated area out of sunlight and away from heat, organic materials, reducing agents and other combustible materials, incompatible materials and foodstuffs.

**KEEP CONTAINERS CLOSED WHEN NOT IN USE** to ensure contamination does not occur - check regularly for leaks. Do not combine part drums of the same product, as this may be a source of contamination. Do not mix with other chemicals. Keep dry - reacts with water, may lead to drum rupture.

**This material is a Schedule Poison 3B and must be stored, maintained and used in accordance with the relevant regulations.**

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

**NATIONAL EXPOSURE STANDARDS:** No value assigned for this specific material by NOHSC, however as published by NOHSC:

**TWA: for Sulphuric Acid = 1 mg/m³**

**S.T.E.L: for Sulphuric Acid = 3 mg/m³**

**DIODELIG LIMIT VALUES:** No biological limit allocated.

**ENGINEERING CONTROLS:** Ensure ventilation is adequate to maintain air concentrations below Exposure Standards. If inhalation risk exists then use with local exhaust ventilation or while wearing suitable respirator. Keep containers closed when not in use.

**Product Name:** SULPHURIC ACID 98%

**Page:** 2 of 2
MATERIAL SAFETY DATA SHEET

PERSONAL PROTECTIVE EQUIPMENT: Protective equipment must be worn at all times. Risk assessments should always be conducted to identify the hazards and in turn determine the appropriate personal protective equipment for the hazard.

Protective glasses: elbow-length laminate film, nitrile, natural rubber, neoprene, neoprene/natural rubber blend or PVC impervious gloves. Always check with the glove manufacturer or your personal protective equipment supplier regarding the correct type of glove to use. Consult AS/NZS 2101 for further information.

Eye protection: safety glasses/poggles with side shield protection and/or full-face shield. Consult AS/NZS 1338 and AS/NZS 1337 for further information.

Clothing and footwear: waterproof spray paints, coveralls, trousers, long-sleeved shirt, closed shoes and/or safety footwear. Consult AS/NZS 2210 and AS/NZS 2919 for further information.

Respiratory Protection: Avoid breathing mist, spray or vapours. Where ventilation is not adequate, respiratory protection may be required. Any air-purifying respirator with an acid gases and vapour filter or any chemical cartridge respirator with an acid gas cartridge(s) providing protection against the compound of concern meeting the requirements of AS/NZS 1715 and AS/NZS 1716.

9. PHYSICAL AND CHEMICAL PROPERTIES:

APPEARANCE: Clear to slightly hazy, colourless, viscous, heavy liquid.

ODOUR: Colourless.

PH (NEUTRAL): <1

SPECIFIC GRAVITY OR DENSITY: 1.40

VAPOUR PRESSURE: <0.001 mm Hg

PERCENT VOLATILES: Approx. 50% w/w

BOILING POINT RANGE: No information available.

FREEZING / MELTING POINT: No information available.

SOLUBILITY: The product is water-based and is fully soluble in water.

FLASH POINT: No known fire hazard.

FLAMMABILITY LIMITS: No information available.

IGNITION TEMPERATURE: No information available.

SHELF LIFE: 2 years from manufacturing date (when stored as directed).

OTHER: None.

10. STABILITY AND REACTIVITY

CHEMICAL STABILITY: Stable under normal conditions of use. The shelf life is 2 years.

CONDITIONS TO AVOID: Do not combine parts of the same product, as this may be a source of contamination.

INCOMPATIBLE MATERIALS: Alkalis, organic materials, aluminium and alloys, cast iron, brasses, tin or zinc coated metals, strong oxidising and reducing agents.

HAZARDOUS DECOMPOSITION PRODUCTS: The packaging material may burn to emit noxious fumes.

HAZARDOUS REACTIONS: Reacts violently with alkalis with evolution of heat. Reacts violently with water and organic materials with the evolution of heat. Sulphuric acid is a strong oxidising agent and reacts violently with combustible and reducing materials. Corrosive to many metals with the liberation of extremely flammable hydrogen gas.

11. TOXICOLOGICAL INFORMATION

No adverse health effects expected if the product is handled in accordance with this Safety Data Sheet and the product label. Symptoms or effects that may arise if the product is mishandled or overexposure occurs are:

ACUTE EFFECTS

INGESTION: Swallowing can result in nausea, vomiting, diarrhoea, abdominal pain and chemical burns to the gastrointestinal tract.

EYE CONTACT: Corrosive to eyes and may injure the cornea. Contamination of eyes can result in permanent injury. Symptoms include itching, tearing, redness and swelling of eyes.

SKIN CONTACT: Corrosive to skin – may cause skin burns. May not produce an immediate burning sensation upon contact, delaying the awareness that contact has occurred. Symptoms may include redness, burning, and swelling of skin, burns, and other skin damage.

Product Name: SULPHURIC ACID 80%
MATERIAL SAFETY DATA SHEET

INHALATION: Mist and vapours are corrosive and dangerous. May irritate or cause tissue damage to respiratory system.

LONG TERM EFFECTS: No information available for the product.

For the component Sulphuric acid: Repeated overexposure may lead to chronic conjunctivitis, lung damage and dental erosion. The International Agency for Research on Cancer (IARC) have concluded that occupational exposure to strong inorganic acid mists containing sulphuric acid is carcinogenic to humans, causing cancer of the lung and to a lesser extent, the liver. No direct link has been established with sulphuric acid, itself, and cancer in humans. Exposure to any mist or aerosol during the use of this product should be avoided and exposure should not exceed the exposure standard.

ACUTE TOXICITY / CHRONIC TOXICITY: No toxicity data for this specific product, however toxicity data for the hazardous ingredient is listed below:

TOXICITY DATA FOR SULPHURIC ACID:
Oral LD50 (rat) 2140 mg/kg Inhalation LC50 (rat) 510 mg/m³/24h
Unreported Route LD50 (man) 136 mg/kg Eye irritation (Rabbit) 5 mg/30s rinse (Severe)

12. ECOLOGICAL INFORMATION

ECOTOXICITY: Avoid contaminating waterways. The product is highly acidic. If large spills occurred a water pH drop could be responsible for an environmental effect on aquatic organisms.

ECOTOXICITY DATA FOR SULPHURIC ACID:
Mosquito fish LC50 42 mg/L/16hr
Flounder fish LC50 80-90 mg/L/48hr
Shore Crab LC50 70 - 80 mg/L/48hr
Cockle LC50 200 - 500 mg/L/48hr

PERSISTENCE AND DEGRADABILITY: Not relevant.

MOBILITY: No information available.

OTHER: None.

13. DISPOSAL CONSIDERATIONS

DISPOSAL METHODS: Empty containers should be forwarded to an approved agent for recycling. Avoid unauthorised discharge to sewer.

SPECIAL PRECAUTIONS FOR LANDFILL OR INCINERATION: The product is suitable for disposal by landfill through an approved agent. Incineration of the product is not recommended, as it is unlikely to adequately burn.

14. TRANSPORT INFORMATION

ROAD AND RAIL TRANSPORT: Classified as Dangerous Goods by the criteria of the Australian Dangerous Goods Code (ADG Code) for transport by Road and Rail.

UN NUMBER: 2796

UN PROPER SHIPPING NAME: SULPHURIC ACID with not more than 51% acid or BATTERY FLUID, ACID

CLASS AND SUBSIDIARY RISK(s): 8

PACKAGING GROUP: II

HAZCHEM CODE: 2R

INITIAL EMERGENCY RESPONSE GUIDE: Guide 37

SEGREGATION DANGEROUS GOODS: Not to be loaded with explosives (class 1), dangerous when wet substances (class 4.3), oxidising agents (class 5.1), organic peroxides (class 5.2), radioactive substances (class 7), corrosives (strong alkaline of class 8), foodstuffs and foodstuff emulsions, however exceptions may apply.

MARINE TRANSPORT: Classified as Dangerous Goods by the criteria of the International Maritime Dangerous Goods Code (IMDG Code) for transport by sea.

UN NUMBER: 2796

UN PROPER SHIPPING NAME: SULPHURIC ACID with not more than 51% acid or BATTERY FLUID, ACID

CLASS AND SUBSIDIARY RISK(s): 8

PACKAGING GROUP: II

STOWAGE AND SEGREGATION: Category B
**MATERIAL SAFETY DATA SHEET**

**AIR TRANSPORT:** Classified as Dangerous Goods by the criteria of the International Air Transport Association (IATA) for transport by air.

**UN NUMBER:** 2796

**UN PROPER SHIPPING NAME:** SULPHURIC ACID with not more than 51% acid

**CLASS AND SUBSIDIARY RISK(S):** 8

**PACKAGING GROUP:** II

**ERG CODE:** 8L

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**15. REGULATORY INFORMATION**

**POISONS SCHEDULE (AUST.):** 6

**APVMA STATUS:** Not relevant.

**TGA STATUS:** Not relevant.

**AICS STATUS:** All the constituents of this product are listed.

**AQIS STATUS:** Not relevant.

**OTHER:** None.

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**16. OTHER INFORMATION**

**GENERAL INFORMATION:** This product is a strong acid. Use good industrial hygiene.

**MSDS ISSUE NUMBER:** 001

**MSDS ISSUE DATE:** 29 June 2003

In any event, the review and, if necessary, the re-issue of a MSDS shall be no longer than 5 years after the last date of issue.

**REASON(S) FOR ISSUE:** Update to conform to requirements of NOHSC:2011(2003); 16-header format.

**THIS ISSUE NUMBER REPLACES ALL PREVIOUS ISSUES.**

**LITERARY REFERENCE:**


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**LEGEND:**

- AICS: Australian Inventory of Chemical Substances
- APVMA: Australian Pesticides and Veterinary Medicines Authority
- AGIS: Australian Quarantine and Inspection Service
- AS: Australian Standard (as issued by Standards Australia)
- ERP Code: Emergency Response Drill Code as found in the ICAO (International Civil Aviation Organisation) Doc 9491
- MSDS: Material Safety Data Sheet
- NOHSC: National Occupational Health and Safety Commission
- STEL: Short Term Exposure Limit - A 15 minute TWA exposure which should not be exceeded at any time during a working day even if the eight-hour TWA average is within the TWA exposure standard. Exposures at the STEL should not be longer than 15 minutes and should not be repeated more than four times per day. There should be at least 60 minutes between successive exposures at the STEL.
- TGA: Therapeutic Goods Administration
- TLV: Threshold Limit Value - TLV is a proprietary name registered by the American Conference of Governmental Industrial Hygienists (ACGIH) and refers to airborne concentrations of substances or levels of physical agents to which it is believed that nearly all workers may be repeatedly exposed day after day without adverse effect.
- TWA: Time Weighted Average - The average airborne concentration of a particular substance when calculated over a normal eight-hour working day, for a five-day working week.

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This MSDS has been prepared from current technical data and summarises at the time of issue our best knowledge of the health and safety information of the product, and in particular how to safely handle and use the product in the workplace.

If clarification or further information is needed to ensure that an appropriate assessment can be made, the user should contact this company.

Our responsibility for products sold is subject to our standard terms and conditions, a copy of which is sent to our customers and is also available upon request.

This MSDS may only be reproduced in full. Summaries or excerpts from this MSDS may not contain all the relevant information and are not permitted.

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*End of MSDS*
APPENDIX 3
Marinova Paper
Pilot clinical study to evaluate the anticoagulant activity of fucoidan
M. R. Irhimeh, J. H. Fitton and R. M. Lowenthal
Pilot clinical study to evaluate the anticoagulant activity of fucoidan
Mohammad R. Irhimeh, J. Helen Fitton and Ray M. Lowenthal

Seaweed-derived heparin-like substances such as fucoidan have been extensively studied in vitro as potential blood anticoagulants. However, there have been no human studies investigating the anticoagulant activity of fucoidan when administered orally. This pilot clinical trial was aimed to assess the safety and clinical effects of fucoidan ingestion on hemostasis as well as study its in-vitro anticoagulant activity. In a single-blinded clinical trial, a total of 20 human volunteers were allocated to both the placebo group (n = 10) who ingested 3 g of guar gum capsules and to the active treatment group (n = 10) who ingested 3 g of 75% fucoidan capsules for 12 days. Platelet indices, activated partial thromboplastin time, antithrombin-III, thrombin time, prothrombin time, and antifactor-Xa were analyzed according to standard methods. In vitro, activated partial thromboplastin time increased from 28.41 to 34.01 s (n = 10, P = 0.01), thrombin time decreased from 18.62 to 17.55 s (n = 10, P = 0.04), and antithrombin-III increased from 113.5 to 117% (n = 10, P = 0.03). The in-vitro fucoidan anticoagulant activity was found prominent. It increased activated partial thromboplastin time, thrombin time, and prothrombin time, whereas antithrombin-III decreased.

In-vivo effect of fucoidan on hemostasis was not obvious probably due to low intestinal absorption. Thus, fucoidan in the form used in this study does not seem to have an oral anticoagulant activity, but it has a very strong in-vitro anticoagulant activity. Blood Coagul Fibrinolysis 20:607–610 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Keywords: activated partial thromboplastin time, anticoagulant, fucoidan, hemostasis, polysaccharides, Undaria pinnatifida

*Clinical Haematology & Medical Oncology Unit, Royal Hobart Hospital, School of Medicine, University of Tasmania, Hobart, Tasmania, Australia. *Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Hashemite University, Zarqa, Jordan and *Marinova Pty. Ltd., Cambridge, Tasmania, Australia

Correspondence to Assistant Professor Mohammad R. Irhimeh, Medical Laboratory Sciences, Faculty of Allied Health Sciences, Hashemite University, Zarqa 13115, Jordan.
Tel: +962 5 390 3933x834; fax: +962 5 390 3308; e-mail: irhimeh@hu.edu.jo

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Accepted 24 July 2009

Introduction

Many marine algal extracts possess anticoagulant properties. About 150 species representing three major divisions of marine algae have been reported to have blood anticoagulant activities [1] with a greater incidence of anticoagulant activity in extracts from the Phaeophyta [2].

The anticoagulant activity of brown marine algae is attributed to "fucans", a class of sulfated fucose-rich carbohydrates, which includes fucoidan, fucoidan, ascosphyllan, sargassan, and glucuronoxyluocan [2]. The most active fucoidan fractions have high fucose content and the thrombin inhibition activity of these fractions exceeds that of heparin [3]. Fucoidan activity is related to its capacity to catalyze the inhibition of thrombin by its natural inhibitors antithrombin and heparin cofactor-II [4,5].

Like heparin, fucoidan are not homogenous substances. The Undaria pinnatifida fucoidan is a galactofucan sulfate with an average molecular weight of approximately 715 kD. The type of linkage is 1→3 and 1→4, and C-2 or C-4 is sulfated.

The widely used naturally occurring anticoagulant heparin has several side effects such as development of thrombocytopenia [6,7], hemorrhagic effect [8,9], ineffectiveness in congenital or acquired antithrombin deficiencies, and incapacity to inhibit thrombin bound to fibrin [10,11]. It is extracted from pig intestine or bovine lung, posing a risk of zoonoses and potential ethical issues, highlighting a need for alternative anticoagulant and antithrombotic therapies, especially ones that are orally available.

Materials and methods

Human volunteers

Twenty healthy human volunteers of either sex aged between 23 and 58 years (average 40) were chosen after advertising and meeting the study criteria. All volunteers were nonsmokers with no history of thyroid abnormalities or sulfur allergy and had not received any medication in the 30 days prior to commencing the study and had not taken food supplements, seafood, or food containing seaweed-derived products in the 7 days prior to commencing the study and during the study. Informed consent was obtained from all volunteers after human ethics approval was obtained from the Southern Tasmania Health & Medical Human Research Ethics Committee and the Royal Hobart Hospital Research Ethics Committee.
The clinical trial is a single-blinded clinical phase I/II trial using a single dose of either guai gum fiber as a placebo treatment or seaweed extract containing 75% fucoidan as an active treatment. Volunteers took 3 g of the treatment three times daily (t.i.d.) for a period of 12 days. Venous blood samples were collected from volunteers using citrate and EDTA anticoagulant-containing tubes for different tests.

**Preparation of capsules**

The study fucoidan is a highly sulfated, polyanionic soluble fiber derived from Tasmanian *U. pinnatifida* (Marinova Pty. Ltd., Hobart, Tasmania, Australia) using Maritech extraction process. It is an off-white powder containing less than 10% (w/w) moisture, 24.76% sucrose, 20.35% galactose, 29.07% sulfate, 2.19% protein, no mannose and 7% bound ions. For placebo, we chose a neutral nonsulfated dietary fiber derived from guar gum (Novartis Pty. Ltd., Mulgrave, Victoria, Australia). The fucoidan and guar gum capsules were prepared as described previously [12].

**Complete blood count and plasma level of fucoidan**

Complete blood counts were obtained for all volunteers before and after taking the fucoidan using an automated cell counter (CELL-DYN 4000 System; Abbott Laboratories, Santa Clara, California, USA). Fucoidan concentration in plasma was measured using the 1B1 antibody, raised against sulfated polysaccharides, according to the competitive enzyme-linked immunosorbent assay (ELISA) method that was described previously [12].

**In-vitro experiments**

For the in-vitro study, 1 g of 75% fucoidan extract was dissolved in 10 ml pooled normal plasma (PNP) to prepare a stock solution at 100,000 mg/l. Then the fucoidan stock solution was diluted with PNP to make serial dilutions ranging from 0 to 50,000 mg/l.

**Coagulation tests**

All tests were performed on the Sysmex CA6000 (Sysmex Corporation, Kobe, Japan) automated instrument using citrated plasma samples. According to the manufacturer's specifications (Dade Behring, Marburg, Germany), the following tests were performed. The activated partial thromboplastin time (aPTT) was determined using Dade Actin FSL activated PTT reagent. The antithrombin-III (AT-III) was determined using Behrichrom Antithrombin-III (A). The thrombin time was determined using thromboclotin assay kit. Anti-factor-Xa (anti-Xa) was determined using spectrolyse heparin (Xa) (Trinity Biotech plc, Bray, County Wicklow, Ireland). The prothrombin time (PT) was quantitatively determined using RecombiPlasTin (Instrumentation Laboratory Company, Lexington, Massachusetts, USA).

**Statistical analysis**

The fucoidan standard curve was constructed using mean and SD. To calculate the fucoidan concentration in the plasma, triplicate readings were averaged and subtracted from the average blank readings. Statistical parameters were calculated using Microsoft Office Excel (Microsoft, Redmond, Washington, USA) and SPSS (SPSS Inc., Chicago, Illinois, USA).

**Results**

**In-vitro hemostasis activity of fucoidan**

It was observed that 75% fucoidan has a very strong hemostasis effect (Table 1). It prolonged the aPTT time from 30.8 s for control to 172.5 s at 63 mg/l. There was no detectable clot formation for concentrations higher than 100 mg/l. The thrombin time was also prolonged but at a higher rate in which it was 15.2 s at baseline and went up to 240.1 s at 15.6 mg/l and then no clot was detected at higher concentrations.

Low concentrations (ranging from 7.8 to 63 mg/l) of the 75% fucoidan had no effect on PT, but at 125 mg/l, the PT began to increase. The prothrombin ratio [international normalized ratio (INR)] was also affected and it increased but not constantly reflecting the changes in the PT. The AT-III decreased with the fucoidan treatment from 108% for control to 89% at 10,000 mg/l. Interestingly, the 73% fucoidan at high levels (10,000–50,000 mg/l) had a strong effect on anti-Xa assay, whereas at low levels the effect was not obvious.

**In-vivo hemostasis activity of fucoidan**

Significant changes were noted in the volunteers' aPTT, thrombin time, and AT-III tests after fucoidan ingestion. In the active treatment group (*n* = 10), the aPTT increased from 28.41 s at baseline to 29.29, 30.15, and 34.01 s after 4, 8, and 12 days (*P* = 0.01), respectively. In the placebo group (*n* = 10), aPTTs were 27.7, 27.5, 27.2, and 27.2 s at 0, 4, 8, and 12 days, respectively. Thrombin

<table>
<thead>
<tr>
<th>Fucoidan (mg/l)</th>
<th>aPTT (s)</th>
<th>TT (s)</th>
<th>AT-III (%)</th>
<th>PT (s)</th>
<th>PR (INR)</th>
<th>Anti-Xa (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (PNP)</td>
<td>30.8</td>
<td>15.2</td>
<td>108</td>
<td>12.8</td>
<td>1.05</td>
<td>0.78</td>
</tr>
<tr>
<td>7.8</td>
<td>40.8</td>
<td>124.5</td>
<td>115</td>
<td>11.3</td>
<td>0.94</td>
<td>0.98</td>
</tr>
<tr>
<td>15.6</td>
<td>53.6</td>
<td>240.1</td>
<td>120</td>
<td>114.4</td>
<td>0.95</td>
<td>0.76</td>
</tr>
<tr>
<td>31</td>
<td>86.0</td>
<td>240.1</td>
<td>120</td>
<td>126.6</td>
<td>1.05</td>
<td>0.77</td>
</tr>
<tr>
<td>63</td>
<td>172.6</td>
<td>240.1</td>
<td>117</td>
<td>142.2</td>
<td>1.18</td>
<td>0.76</td>
</tr>
<tr>
<td>125</td>
<td>340.1</td>
<td>240.1</td>
<td>120</td>
<td>118.2</td>
<td>1.5</td>
<td>0.78</td>
</tr>
<tr>
<td>250</td>
<td>340.1</td>
<td>240.1</td>
<td>118</td>
<td>26</td>
<td>2.17</td>
<td>0.79</td>
</tr>
<tr>
<td>500</td>
<td>340.1</td>
<td>143.3</td>
<td>115</td>
<td>45.5</td>
<td>3.78</td>
<td>0.78</td>
</tr>
<tr>
<td>1000</td>
<td>340.1</td>
<td>240.1</td>
<td>114</td>
<td>120.1</td>
<td>1.01</td>
<td>0.80</td>
</tr>
<tr>
<td>5000</td>
<td>340.1</td>
<td>240.1</td>
<td>132</td>
<td>120.1</td>
<td>1.01</td>
<td>0.80</td>
</tr>
<tr>
<td>10,000</td>
<td>340.1</td>
<td>240.1</td>
<td>129</td>
<td>120.1</td>
<td>1.01</td>
<td>0.85</td>
</tr>
<tr>
<td>25,000</td>
<td>340.1</td>
<td>240.1</td>
<td>63</td>
<td>120.1</td>
<td>1.01</td>
<td>0.52</td>
</tr>
<tr>
<td>50,000</td>
<td>340.1</td>
<td>240.1</td>
<td>40</td>
<td>120.1</td>
<td>1.01</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Each value in the table represents the average of three independent duplicate experiments. aPTT, activated partial thromboplastin time; AT-III, antithrombin-III; INR, international normalized ratio; PNP, pooled normal plasma; PT, prothrombin ratio; PT, prothrombin time; TT, thrombin time.

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Table 2  Different coagulation tests’ results for healthy volunteers who ingested 3 g of guar gum as a placebo group or 3 g of 75% fucoidan

<table>
<thead>
<tr>
<th></th>
<th>TT Ref (14–17 s)</th>
<th>AT-III Ref (90–140 %)</th>
<th>PT time Ref (11–13 s)</th>
<th>PR (INR) Ref &lt;1.2</th>
<th>LMWH (Anti-Xa activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo volunteers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>16.23 ± 0.22</td>
<td>119 ± 4.13</td>
<td>11.88 ± 0.26</td>
<td>0.97 ± 0.03</td>
<td>0</td>
</tr>
<tr>
<td>4 Days</td>
<td>16.26 ± 0.17</td>
<td>118 ± 0.24</td>
<td>11.96 ± 0.26</td>
<td>0.98 ± 0.03</td>
<td>0</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Fucoidan-treated volunteers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>16.32 ± 0.69</td>
<td>113 ± 2.51</td>
<td>11.54 ± 0.17</td>
<td>0.95 ± 0.02</td>
<td>0</td>
</tr>
<tr>
<td>4 Days</td>
<td>17.25 ± 0.20*</td>
<td>117 ± 2.90*</td>
<td>11.85 ± 0.17</td>
<td>0.99 ± 0.02</td>
<td>0</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Results are average ± mean SE. AT-III, antithrombin-III; INR, international normalized ratio; LMWH, low-molecular-weight heparin; PT, prothrombin time; Ref, reference; TT, thrombin time. *P value ≤0.05.

Time decreased significantly from 18.62 s at baseline to 17.55 s after 4 days (n = 10, P = 0.04), and in contrast to the in-vivo data AT-III increased significantly from 113.5% at baseline to 117% after 4 days (n = 10, P = 0.02; Table 2). We did not look at the thrombin time and AT-III levels after 8 or 12 days of capsules ingestion. For other parameters, PT and prothrombin ratio (INR), nonsignificant changes were noted and anticoagulant-Xa was not affected under the implemented treatment conditions (Table 2).

In comparison with the placebo group, there were no significant changes in any of platelet indices when volunteers ingested the 75% fucoidan daily for up to 12 days. The placebo group (n = 10) baseline reading and readings after 4, 8, and 12 days were for platelet count 327, 333, 313, and 306/μl; for the mean platelet volume (MPV) 8.96, 8.77, 8.79, and 8.52; and for the platelet distribution width (PDW) 16.23, 15.87, 16.00, and 16.10, respectively, whereas the 75% fucoidan-group (n = 10) baseline reading and readings after 4, 8, and 12 days were for platelet count 280, 279, 285 and 285/μl; for the MPV 8.29, 8.29, 8.09 and 8.00; and for the PDW 16.07, 15.90, 15.81, 16.03, respectively.

Detection of plasma level of fucoidan after oral administration

After ingesting the capsules, the median concentration of fucoidan in volunteers’ plasma for the placebo group was 0.17 mg/l (confidence interval mean (CIM) 0.02; n = 10), whereas it was 13.06 mg/l (CIM 0.03; n = 10) for the fucoidan-treated group.

Discussion

In this study, orally administered fucoidan prolonged the aPTT from 28.41 s at baseline to 34.01 s after 12 days (n = 10, P = 0.01; average change at 5.6 s), which indicates that fucoidan may alter a specific part of the intrinsic coagulation pathway. The significant changes in thrombin time and AT-III are consistent with this. aPTT may also detect severe functional changes in factors II, V, X, or fibrinogen. It has been widely used to monitor the effectiveness of heparin therapy. Engelberg [13] has shown that there was a small significant effect on aPTT averaged at 2.5 s after administering 20,000 units of oral heparin to 45 humans. Hiebert et al. [14] have also observed in an HIV human trial an increase in the aPTT levels after long-term oral administration of dextran sulfate (8000 Da, 1 g four times daily). These observations are of a similar magnitude to those noted in this study with oral fucoidan. The small increase in aPTT is unlikely to be clinically valuable for acute therapeutic use, but there are implications for possibly including this algal extract in the diet in an ongoing manner.

Orally administered heparin had antithrombotic activity between 50 and 100% in rat models [15]. This was associated with the presence of about 1% of the orally administered heparin dose in plasma. Similarly, our findings indicate that the average level of fucoidan in plasma was 0.3% of the oral dose with plasma levels of about 13.06 mg/l [12]. It is possible that fucoidan has heparin-like action (i.e. to prolong aPTT via anti-Xa and anti-IIa activity).

Circulating levels of factors II, VII, XI, and X are depressed in patients receiving oral anticoagulants [16]. In vivo, there was a nonsignificant increase in the PT. This finding may suggest that fucoidan in the dose used may have little effect on the extrinsic mechanism of coagulation, including no effect on the activity of factors I, II, V, VII, and X. The clinical trial showed no effect of the fucoidan on the anti-Xa assay; however, the in-vitro assay showed that fucoidan at high levels (>10 mg/ml) has an effect on anti-Xa assay. This may suggest that the major mechanism of action is with thrombin rather than factor Xa, which may provide an insight into the mechanism of action of fucoidan.

The in-vitro assays in this study showed that fucoidan has the ability to decrease the AT-III assay in a concentration-dependent manner. The in-vitro thrombin time assay showed that the fucoidan has the ability to alter the last part of the coagulation pathway in a concentration-dependent manner. Prolonging of thrombin time is also seen with heparin therapy and in patients with dysfibrinogenemia or fibrinogenemia. The increase in the thrombin time in vitro may reflect an inhibitory effect on fibrinogenolysis, which is not seen in vivo.

Indeed, the thrombin time, the most sensitive assay to fucoidan in vitro, actually shortened following oral administration. This indicates that fucoidan has the ability to

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interfere with the final stage of the coagulation process. Fibrinogen levels were not tested in this study however; the results from the in-vitro study may indicate that fucoidan interferes with the fibrin production, thereby preventing the formation of the clot or inhibiting thrombin-mediated conversion of fibrinogen to fibrin. The discordance between the in-vivo and in-vitro results suggests that the circulating material measured as fucoidan might have lacked a significant proportion of its anticoagulant activity.

We did not observe an effect of the fucoidan on blood platelet count, but an effect on platelet aggregation has not been excluded. Studies have shown that fucoidans can enhance platelet aggregation in vitro. However, this effect was found to depend on the fucoidan molecular weight [17]. Another study on baboons found fucoidan to be a potent inhibitor of platelet aggregation in vivo [18]. Platelet function and fibrinolysis have not been assessed in this study.

In conclusion, this study demonstrated that a small quantity of bioavailable fucoidan, given orally, had a modest but significant effect on some of the coagulation assays, in particular, the intrinsic pathway. Changes in the coagulation tests were still within reference ranges and unlikely in themselves to be 'clinically valuable'. However, this study demonstrates the potential of a more bioavailable form of oral fucoidan or for subcutaneous or intravenous forms.

Acknowledgement
We would like to thank Marinova Pty. Ltd., Australia, for kindly providing the fucoidan extract as a gift and Professor David Kilpatrick and Dr James Daly for their useful comments and to the RHH staff, especially Belinda Snooks. J.H.F. is employed by Marinova as a senior research scientist.

References
Fucoidan ingestion increases the expression of CXCR4 on human CD34+ cells
M. R. Irhimeh, J. H. Fitton, and R. M. Lowenthal
Fucoidan ingestion increases the expression of CXCR4 on human CD34+ cells

Mohammad R. Ihimehan, J. Helen Fitton, and Raymond M. Lowenthal

Objective. Transplantation of hematopoietic progenitor stem cells (HPC) is an important treatment modality for a variety of neoplastic diseases. HPC collection for transplantation with granulocyte colony-stimulating factor may be unsuccessful in patients who have received prior chemotherapy or for other reasons. Methods to improve mobilization of HPCs are required. Disruption of the interaction between the cell surface receptor CXCR4 and its ligand stromal derived factor-1 (SDF-1) is a mechanism for HPC release from the bone marrow into the peripheral blood (PB).

Methods. We carried out a clinical trial to evaluate the effects of ingestion of a fucoidan, galactofuran sulfate (a putative HPC mobilizing agent) on circulating CD34+ cells, CXCR4 expression, and levels of SDF-1, interferon gamma (IFN-γ) and interleukin 12.

Results. Following ingestion of fucoidan, CD34+ cells increased significantly in the PB from 1.64 to 1.84 cells/μL after 4 days. The proportion of CD34+ cells that expressed CXCR4 increased from 45 to 90% after 12 days, the plasma level of SDF-1 increased from 1978 to 2010 pg/mL, and IFN-γ level increased from 9.04 to 9.89 pg/mL.

Conclusion. Oral fucoidan significantly amplified the CXCR4+ HPC population. The ability to mobilize HPC using sulfated polysaccharides and mobilize more HPC with high levels of CXCR4 could be clinically valuable. © 2007 International Society for Experimental Hematology. Published by Elsevier Inc.

Autologous transplants of hematopoietic progenitor stem cells (HPC) are used to treat a variety of neoplastic and other diseases. HPC can be mobilized from the bone marrow (BM) niche into the peripheral blood (PB) via the administration of granulocyte colony-stimulating factor (G-CSF) or other agents such as AMD3100 or fucoidan. The mechanisms for the mobilization may include modulation of serine proteases, metalloproteases, and of stromal derived factor-1 (SDF-1)-CXCR4 interactions [1].

The success of subsequent engraftment may be associated with the expression of the receptor CXCR4 on CD34+ HPC. This effect can be observed both clinically [2] and experimentally [3,4]. Mobilization of adequate amounts of HPCs including CD34+ CXCR4+ is not always successful. Failure to achieve sufficient mobilization can occur in patients who have received multiple cycles of chemotherapy or for other, unknown reasons.

Previous research has shown that intravenous (IV) fucoidan has a pronounced and extended mobilizing effect on HPC in mice and on nonhuman primates [5–8]. This effect is postulated to be the result of dissociation of the chemokine SDF-1 from the BM stroma, creating an attractive gradient into the peripheral circulation. Disruption of the interaction between the CXCR4 and its ligand SDF-1 is one of the mobilizing mechanisms also common to G-CSF [9] and newer agents such as AMD3100 [10,11].

Fucoidan is a generic term for the sulfated, fucose-rich polysaccharides derived from brown macroalgae [12] or echinoderms [13]. In animal models, ingestion of fucoidan has inhibitory effects on tumors, which appear to be associated with a rise in internerferon-gamma (IFN-γ), interleukin-12 (IL-12), and stimulation of innate immunity [14–17]. In vitro treatment of BM mononuclear cells (MNCs) with IFN-γ can upregulate the expression of CXCR4 granulocyte precursors and monocytes [17].

Despite the available literature evidence that IV fucoidan had an effect in tumor animal models and mobilization of HPC, there are no reports to date about the clinical use of oral fucoidan to modulate or mobilize HPC. Although
fucoidans synergize with G-CSF to increase mobilization 11 times over G-CSF alone in primates, to date no clinical trials in patients have been reported. In this study, we examined the effects of orally ingested Undaria pinnatifida fucoidan on the PB stem cells, the expression of CXCR4, and plasma levels of SDF-1, IL-12, and IFN-γ.

Materials and methods

Human volunteers
In a single-blind, randomized, placebo-controlled clinical trial, 37 nonsmoker volunteers of either sex were divided into three groups after giving informed consent and after human ethics approval was obtained from the Southern Tasmania Health & Medical Human Research Ethics Committee. As placebo, six volunteers took 3 g of guar gum. Another six volunteers took 3 g of whole Undaria containing 10% w/w fucoidan, and another 25 volunteers took 3 g of 75% w/w fucoidan daily for 12 days. All volunteers took three capsules (0.33 g each) three times a day. During the study time, volunteers were asked not to eat any seafood, seaweed-derived products, drugs, or food supplements. Blood samples were collected as described later.

Preparation of capsules
Fucoidan is a highly sulfated, polyanionic soluble fiber derived from the brown seaweed Undaria pinnatifida by Marinova Pty. Ltd. (Hobart, TAS Australia). We chose a neutral nonsulfated dietary fiber derived from guar gum as a placebo (Novartis Pty. Ltd., Mulgrave, VIC Australia). The capsules were prepared as described previously [15]. The structure and the therapeutic characteristics of these compounds have been described previously [12,14,15].

Collection of blood samples
Venous blood from the antecubital vein from the three groups of volunteers was collected using ethylenediamine-tetraacetic acid (EDTA) tubes. Plasma samples (platelet poor for SDF-1 assay) were collected and stored in aliquots at −80°C within 30 minutes of collection for later analysis. Complete blood counts were obtained using an automated cell counter (CELL-DYN-4000 System, Abbott Lab., IL, USA).

Flow cytometry analysis
The expression of different surface membrane markers on normal human PB HPCs was evaluated by fluorescein-activated cell sorting (FACS) direct immunofluorescence. Cells were Fc-blocked with 1 μg of human immunoglobulin G (IgG)/10^6 cells (Zymed Lab., San Francisco, CA, USA) for 15 minutes at room temperature, and then stained with the designated monoclonal antibody. FITC-CD34, Cy5-CD45 (Becton Dickinson, San Jose, CA, USA) and PE-CXCR-4 (R&D Systems Inc., Minneapolis, MN, USA) were used in the study. A negative control tube was prepared identically but contained isotype controls IgG1, or IgG2a antibodies (BD). FACS-fixed cells were analyzed using FACScan flow cytometer and the Cell Quest software package (BD).

Preparation of MNCs and colony-forming unit granulocyte assays
Human PB MNCs were isolated from healthy subjects using Histopaque-1077 kit and protocol from Sigma-Aldrich Co. (St. Louis, MO, USA). Briefly, 3 mL of EDTA blood was layered onto the Histopaque-1077 and centrifuged at 400g for 30 minutes. The opaque interface was mixed with 10 mL isotonic phosphate-buffered saline (PBS) then centrifuged at 250g for 10 minutes. The cell pellet was washed with 5 mL PBS twice and resuspended in 0.3 mL PBS. Peripheral blood mononuclear cells were plated at densities of 1 × 10^5 viable nucleated cells per 35-mm plate using growth medium (MethoCult GFTh4534, StemCell Technologies, Vancouver, BC, Canada), then colony-forming unit granulocyte (CFU-GM) was performed. Petri dishes were incubated for 14 days at 37°C in a humidified atmosphere containing 5% CO2. CFU-GM colonies defined as clusters of ≥30 cells were then counted using an inverted microscope and recorded as the mean of quadruplicate counts. Plates were neglected if contaminated or > 50 colonies were counted.

Plasma cytokines and cytokine assays
PB was collected from volunteers according to the designated schedule in tubes containing EDTA. Platelet-poor plasma was prepared within 30 minutes of blood collection and stored at −80°C for later analysis. Three different cytokines, SDF-1, IFN-γ, and IL-12 levels were analyzed directly after thawing plasma samples gradually on the day of test by enzyme-linked immunosorbent assay using kits and protocols from R&D Systems Inc.

Statistical analysis
Student’s t-test and analysis of variance were used to analyze data. A p value of 0.05 was chosen as the limit of statistical significance. Triplicate readings for each sample were averaged. All other statistical parameters were calculated using Microsoft Office, Excel and SPSS, version 12.

Results
No side effects were reported, and none of the volunteers exhibited toxicity when 3 g of guar gum, 10% fucoidan, or 75% fucoidan extracts were taken orally three times a day for 12 days.

Fucoidan ingestion caused mild leukopenia and lymphopenia but had no effect on neutrophils.

We observed a nonsignificant decrease in the total number of leukocytes in the PB when 10% fucoidan was ingested, but when 75% fucoidan was ingested, the decrease was significant after 12 days (Table 1). There was a decrease in leukocytes from 5.74 cells/nL at baseline to 5.37 after 12 days (p = 0.05). Of the leukocyte fractions, lymphocytes were most affected. Ingestion of either 10% or 75% fucoidan decreased the lymphocyte count but the decrease was only significant with the 75% fraction. The absolute number of lymphocytes decreased from 2.18 cells/nL at baseline to 1.98 after 12 days (p = 0.03; Table 1). Neutrophil count was not affected after ingesting guar gum or 10% or 75% fucoidan. Furthermore, there was no effect on the expression of CD16 in the CD45+ population (results not shown).
Table 1. Average readings of all of the tests at four time points

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Test</th>
<th>0 days ±</th>
<th>4 days ±</th>
<th>8 days ±</th>
<th>12 days ±</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gum</td>
<td>Leukocyte (cells/μL)</td>
<td>7.37 ± 0.23</td>
<td>7.38 ± 0.61</td>
<td>7.03 ± 0.87</td>
<td>7.22 ± 0.71</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte (cells/μL)</td>
<td>2.80 ± 0.13</td>
<td>2.82 ± 0.16</td>
<td>2.65 ± 0.15</td>
<td>2.83 ± 0.18</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Neutrophil (cells/μL)</td>
<td>3.63 ± 0.44</td>
<td>3.52 ± 0.38</td>
<td>3.45 ± 0.32</td>
<td>3.43 ± 0.26</td>
<td>6</td>
</tr>
<tr>
<td>IFN-γ (pg/μL)</td>
<td>9.03 ± 0.18</td>
<td>9.14 ± 0.15</td>
<td>9.2 ± 0.15</td>
<td>9.17 ± 0.08</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>10% fucoidan</td>
<td>Leukocyte (cells/μL)</td>
<td>7.48 ± 0.35</td>
<td>6.98 ± 0.26</td>
<td>7.01 ± 0.51</td>
<td>6.95 ± 0.59</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte (cells/μL)</td>
<td>2.87 ± 0.12</td>
<td>2.73 ± 0.16</td>
<td>2.6 ± 0.30</td>
<td>2.62 ± 0.26</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Neutrophil (cells/μL)</td>
<td>3.73 ± 0.28</td>
<td>3.42 ± 0.18</td>
<td>3.42 ± 0.24</td>
<td>3.35 ± 0.31</td>
<td>6</td>
</tr>
<tr>
<td>IFN-γ (pg/μL)</td>
<td>9.01 ± 0.13</td>
<td>9.01 ± 0.38</td>
<td>9.05 ± 0.19</td>
<td>8.97 ± 0.27</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>75% fucoidan</td>
<td>Leukocyte (cells/μL)</td>
<td>5.74 ± 0.28</td>
<td>5.62 ± 0.33</td>
<td>5.48 ± 0.30</td>
<td>5.37 ± 0.37</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte (cells/μL)</td>
<td>2.18 ± 0.14</td>
<td>2.06 ± 0.13</td>
<td>1.95 ± 0.14</td>
<td>1.98 ± 0.14</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Neutrophil (cells/μL)</td>
<td>2.94 ± 0.19</td>
<td>2.97 ± 0.22</td>
<td>2.90 ± 0.19</td>
<td>2.82 ± 0.22</td>
<td>25</td>
</tr>
<tr>
<td>SDF-1 (pg/μL)</td>
<td>1978 ± 25</td>
<td>1996 ± 31</td>
<td>2101 ± 33</td>
<td>2093 ± 47</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>IFN-γ (pg/μL)</td>
<td>9.04 ± 0.42</td>
<td>9.41 ± 0.49</td>
<td>9.89 ± 0.39</td>
<td>9.82 ± 0.57</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

Table shows the mean readings observed on baseline (0 day) and on 4th, 8th, and 12th day after ingesting 3 g of each treatment. Gum is used as a placebo control. All values are average ± mean standard error. n = number of volunteers, different volunteers have been used for each treatment.

*Volunteers ingested 3 g of each treatment three times daily.

The mean value at 0 day (baseline) was used in the t-test as first set of data to which other groups are compared.

1p < 0.05 using paired Student's t-test.

2p < 0.01 using paired Student's t-test.

Increase in CD34+ cell count in PB after fucoidan ingestion

A slight increase in the circulating CD34+ was observed after ingesting fucoidan. When 10% fucoidan (3 g/d) was ingested, a nonsignificant increase in CD34+ in PB, from 1.07 to 1.29 cells/μL (p = 0.06, n = 6) after 12 days, was observed. However, when 75% fucoidan was ingested, the CD34+ count increased from 1.64 cells/μL to 1.84, 1.80, and 1.79 cells/μL at 4, 8, and 12 days, respectively. This increase was significant at day 4 (p = 0.04). Some volunteers presented a large increase in the CD34+ on days 8 and 12 but were considered by the statistical program as outliers and were not included in the calculated median.

Increase in the expression of CXCR4 on CD34+ cells after fucoidan ingestion

When 3 g/d of 10% fucoidan was ingested, a nonsignificant increase in the CD34+CXCR4+ was observed. However, when 3 g/d of the 75% fucoidan was ingested, the CD34+CXCR4+ count increased significantly (p < 0.0002) from 0.75 cells/μL at baseline to 1.65 cells/μL after 12 days (Fig. 1). The proportion of CD34+CXCR4+ increased from 45 to 90% after 12 days of treatment (Table 2). A few volunteers showed a large increase in the CD34+ cell count but were considered by the statistical program as outliers and were not included in the calculated median.

Fucoidan ingestion has no effect on PB MNCs in CFU-GM

Generally, there was a nonsignificant decrease in the number of CFU-GM per microliter of PB blood after ingesting the 75% fucoidan. The mean CFU-GM count at baseline was 1.87/μL (±mean standard error = 0.29) and decreased to 1.71 (±0.26), 1.4 (±0.29), and 1.31 (±0.35) after 4, 8, and 12 days (p = 0.50, 0.19, 0.14; n = 13) of ingesting 75% fucoidan, respectively.

Figure 1. Total number of PB CD34+/CXCR4+ cells at baseline and after 4, 8, and 12 days of taking 75% fucoidan. Black lines represent medians for 23 duplicate experiments representing 23 volunteers. The boxes represent the median (80% of the population), and the error bars represent the mean standard error. +, outliers or high responders.
Table 2. Average percentage of cells that are CXCR4⁺ out of the total CD34⁺ cells at four time points

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>0 days³</th>
<th>4 days</th>
<th>8 days</th>
<th>12 days</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guar gum</td>
<td>62.33 ± 4.64</td>
<td>64.15 ± 2.81</td>
<td>66.43 ± 3.18</td>
<td>63.66 ± 3.09</td>
<td>6</td>
</tr>
<tr>
<td>10% fucoidan</td>
<td>67.27 ± 8.33</td>
<td>65.49 ± 9.13</td>
<td>71.78 ± 9.01</td>
<td>71.96 ± 13.09</td>
<td>6</td>
</tr>
<tr>
<td>75% fucoidan</td>
<td>49.75 ± 7.19</td>
<td>84.57 ± 4.74¹</td>
<td>84.4 ± 5.57¹</td>
<td>91.15 ± 3.63¹</td>
<td>23</td>
</tr>
</tbody>
</table>

The table shows the average readings observed on baseline (0 day) and on 4, 8, and 12 days after ingesting 3 g of each treatment. Guar gum is used as a placebo control. Values shown are average % of CD34⁺/CXCR4⁺ ± mean standard error; n = number of volunteers; different volunteers have been used for each treatment.

*Volunteers ingested 3 g of each treatment three times daily.
³The mean value at 0 days (baseline) was used in the t-test as first set of data.
¹p < 0.01 using paired Student’s t-test.

Increase in plasma levels of SDF-1 and IFN-γ with fucoidan ingestion

Ingestion of guar gum and 10% fucoidan did not affect the levels of SDF-1 and IFN-γ in plasma (Table 1). Volunteers who ingested 3 g of the 75% fucoidan had an elevation in the plasma level of SDF-1 after 8 days from 1978 to 2101 pg/mL (p = 0.00005).

Further, the plasma level of IFN-γ was assessed because this cytokine has been associated with the upregulation of CXCR4. Volunteers who ingested 3 g of the 75% fucoidan showed a significant elevation (p = 0.04) in the plasma IFN-γ, from 9.04 to 9.82 pg/mL (Table 1). However, there was no detectable change in the IL-12 plasma (results not shown).

Discussion

We carried out this clinical study to determine the effects of ingested Undaria-derived fucoidan on PB. We found that there was a small increase in CD34⁺ cells and a profound increase, from 40 to 90%, in the proportion of CD34⁺/CXCR4⁺ when 75% fucoidan was ingested. A smaller increase was noted when 10% fucoidan was ingested. We also observed a significant increase in IFN-γ and SDF-1 in the 75% fucoidan group but not in the control or 10% groups. We did not observe an increase, but rather a nonsignificant decrease in CFU-GM, despite the increase in the CD34⁺ cell count. This slight increase after oral intake contrasts with the large and sustained response elicited by IV fucoidan [5–8], AMD3100 [10], or G-CSF [2].

Previous studies have shown that IV fucoidan produces rapid mobilization of murine HPCs with long-term BM repopulating potential in mice and nonhuman primates [6–9]. CXCR4 was not assessed in those studies. Fucoidan has also been used as a tool to examine the effects of binding SDF-1. Mavier and colleagues [16] demonstrated that after experimental hepatic destruction, IV fucoidan blocked the SDF-1 expression of liver stem cells and markedly decreased their accumulation. Ingestion of fucoidan has been shown to inhibit tumors of various kinds [17], an effect that may be attributable to a stimulation of the nonspecific immune system [14,18]. Fucoidans are well-known experimental selectin blockers. In vitro, the binding of L-selectin on lymphocytes by fucoidan enhanced the expression of CXCR4 in lymphocytes [19]. Clinical use of a fucoidan preparation was made in the 1960 s. Claudio and Stendardo [20] reported favorable results from patients with leukopenia and leukocytosis with increases in the general condition of patients.

In a prior work, two preparations of fucoidan to mobilize HPCs have been used; 100 mg/kg IV of sulfated linear fucan from the sea urchin Lytechinus variegatus [6,8] and 25 mg/kg intraperitoneally of branched fucoidan fraction from Ascoplyium nodosum (from Fluka) [5]. Despite the differences in fucoidans, similar mobilizations were seen and attributed to the creation of SDF-1 gradient into the PB.

The fucoidan used in our studies is derived from Undaria pinnatifida. Our previous work using an antibody-based detection method indicated that when 3 g of 75% fucoidan was ingested daily, plasma concentration was elevated up to 4 mg/L after 4 days, and then 13 mg/L after 12 days despite the fact that fucoidan is a large-molecular-weight material [15]. Acidic conditions in the stomach may cause a limited hydrolysis of the fucoidan. Humans do not produce enzymes capable of breaking down fucoidans, and the latter also appear to be unaffected by human fecal flora [21]. We hypothesized that small quantities of fucoidan may cross the intestinal wall as whole molecules probably by endocytosis. The slight but significant changes in HPCs shown here do not indicate stand-alone utility of this substance as a clinical entity but instead indicate a need for further investigation of its potential. Further work is needed to investigate the repopulating potential of the cells, and the timing of peak values for CXCR4 and total numbers of cells.

AMD3100, which is a reversible inhibitor of the binding of SDF-1α to its cognate receptor CXCR4, is currently in clinical trials as a mobilizing agent. It significantly improves the mobilization capacity of G-CSF when used in combination with it in mice [22]. We postulate that fucoidan may have a similar mechanism of action on SDF-1/CXCR4 that could play a role in the mobilization of CD34⁺ cells from BM to PB especially if fucoidan is used IV.
The increase in CD34⁺CXCR4⁺ cells was marked in this study, although the increase in the CD34⁺ cells was small. Although we observed no significant change in SDF-1 levels at 4 days, when CD34 levels increased, SDF-1 levels were increased by day 12. Either the rise in SDF-1 is not correlated with the enhanced CD34⁺ cell numbers or CXCR4 expression, or the effect is only at the BM level at the 4 day stage.

Previously, CXCR4 expression on HPCs has been shown to increase after administration of G-CSF within both human and murine BM, reaching peak levels at the time of mobilization [9], although SDF-1 levels did not rise. Interestingly, we observed a small decrease in CFU-GM over 12 days in the 75% fucoidan group. This effect may perhaps be attributed to the rise in IFN-γ. Constitutive expression of low levels of IFN-γ by stromal cells has been noted to have a profound inhibitory effect on hematopoiesis [23].

CXCR4 plays an important role in regulating the trafficking of HPCs and their homing/retention in BM, and it modulates several biologic processes in more differentiated cells [24].

In this study, a small, significant decrease in leukocytes and lymphocytes was observed after 12 days of ingesting the 75% fucoidan, although this decrease was within normal clinical range. In previous studies, there was a fall in circulating leukocytes immediately after G-CSF was given [25–27].

The presence of normal number of functional neutrophils is important for mobilization of HPCs [28,29]. In this study, we have demonstrated that 3 g of oral fucoidan has no effect on neutrophil count and does not cause neutropenia.

We observed that there was an increase in the plasma level of IFN-γ, consistent with a previous study that showed an increase in HPCs accompanied by an increase in the level of SDF-1, IFN-γ, and IL-12 [8]. The level of IL-12 in our study did not change (results not shown). It was shown previously that in vitro treatment of BM MNCs with IFN-γ can upregulate the expression of CXCR4 on granulocyte precursors and monocytes [30]. This may, in part, reflect our observation of increased expression of CXCR4 on CD34⁺ cells. It could be more relevant to look at the BM cytokine levels as well and compare the changes between PB and BM levels especially as PB SDF-1 may originate from the BM pool.

In summary, oral administration of fucoidan significantly amplified the CXCR4⁺ HPC population. The ability to mobilize HPCs with high levels of CXCR4 expression could be clinically valuable. However, the effect of IV fucoidin in humans remains to be determined.

Acknowledgments
We would like to thank Marinova Pty. Ltd., Hobart, TAS Australia, for kindly providing the Undaria fucoidin extracts as a gift. Dr. Scott Rapp advised on the methodology, and Dr. Greg Woods kindly assisted in proofreading the manuscript. M.R.I. is a Ph.D. candidate at University of Tasmania, and this work will be submitted in partial fulfillment of the requirement for the Ph.D.

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APPENDIX 5
Marinova paper
A combined phase I and II open label study on the effects of a seaweed extract nutrient complex on osteoarthritis
Biologics: Targets & Therapy 2010, 4, 33 – 44.
A combined phase I and II open label study on the effects of a seaweed extract nutrient complex on osteoarthritis

Stephen P Myers,1,2 Joan O’Connor,1,2 J Helen Fitton,3 Lyndon Brooks,4 Margaret Rolfe,4 Paul Connellan,3 Hans Wohlmut,1,5,6 Phil A Cheras,1,2 Carol Morris3

1NatMed-Research, 2Centre for Health and Wellbeing, 3Graduate Research College, 4Centre for Phytochemistry and Pharmacology, 5Medical Plant Herbarium, Southern Cross University, Lismore, NSW, Australia; 6Marinova Pty Ltd, Hobart, Tasmania, Australia

Background: Isolated fucoidans from brown marine algae have been shown to have a range of anti-inflammatory effects.

Purpose: This present study tested a Maritech® extract formulation, containing a blend of extracts from three different species of brown algae, plus nutrients in an open label combined phase I and II pilot scale study to determine both acute safety and efficacy in osteoarthritis of the knee.

Patients and methods: Participants (n = 12, five females [mean age, 62 ± 11.06 years] and seven males [mean age, 57.14 ± 9.20 years]) with a confirmed diagnosis of osteoarthritis of the knee were randomized to either 100 mg (n = 5) or 1000 mg (n = 7) of a Maritech® extract formulation per day. The formulation contained Maritech® seaweed extract containing Fucus vesiculosus (85% w/w), Macrocystis pyrifera (10% w/w) and Laminaria japonica (5% w/w) plus vitamin B6, zinc and manganese. Primary outcome was the average comprehensive arthritis test (COAT) score which is comprised of four sub-scales: pain, stiffness, difficulty with physical activity and overall symptom severity measured weekly. Safety measures included full blood count, serum lipids, liver function tests, urea, creatinine and electrolytes determined at baseline and week 12. All adverse events were recorded.

Results: Eleven participants completed 12 weeks and one completed 10 weeks of the study. Using a multilevel linear model, the average COAT score was reduced by 18% for the 100 mg treatment and 52% for the 1000 mg dose at the end of the study. There was a clear dose response effect seen between the two treatments (P ≤ 0.0005) on the average COAT score and each of the four COAT subscales (pain, stiffness, difficulty with physical activity and overall symptom severity) (P ≤ 0.05). The preparation was well tolerated and the few adverse events were unlikely to be related to the study medication. There were no changes in blood parameters measured over the course of the study with the exception of an increase in serum albumin which was not clinically significant.

Conclusion: The seaweed extract nutrient complex when taken orally over twelve weeks decreased the symptoms of osteoarthritis in a dose-dependent manner. It was demonstrated to be safe to use over the study period at the doses tested. The efficacy of the preparation now needs to be demonstrated in a phase III randomized controlled trial (RCT).

Keywords: fucoidan, osteoarthritis, complementary medicine, inflammation, TNF alpha

Introduction
Osteoarthritis (OA) is the most frequent cause of disability among adults in the developed world. Arthritis affects around three million people in Australia, representing about 15% of the population. Similarly, more than 20 million people in the United States have the disease. The lifetime risk of knee OA for males and females aged over...
45 years in Johnston County is estimated at between 44.7% (nonobese) and 67% (obese) and these figures are believed to reflect the incidence of OA across the United States.\(^3\) OA costs more than $60 billion per year in the USA and is second only to ischemic heart disease as a cause of work disability in men aged over 50 years.\(^4\) The progressive deterioration of articular cartilage which occurs in OA results in pain, stiffness and difficulty with physical activities. The disease is managed rather than cured with a focus on pain relief.\(^5\) A number of herbal medicines have been found to have beneficial effects in alleviating the symptoms of OA in human clinical studies. These include advocate, soybean unsaponifiables,\(^6\) lipids from green-lipped mussels,\(^7\) calcified seaweed extracts,\(^8\) and Pycnogenol (French maritime pine bark extract).\(^9\) Boswellia serrata extracts have also show clinical promise\(^10\) as do preparations of Harpagophytum procumbens (Devil's Claw).\(^11,12\) Polyphenols such as epigallocatechin (from green tea) and phlorotannin-rich extracts of the seaweed Ecklonia cava have exhibited potential using in vitro models yet have generated little clinical evidence to date.\(^13\)

In the Western herbal medicine tradition, bladderwrack (Fucus vesiculosus) and other seaweeds in the form of topically applied liniments have been used as herbal approaches to the treatment of sore knees.\(^14\) Seaweed extracts have been demonstrated to contain at least two major components with anti-inflammatory activity: fucophans and polyhaloroglucinos (algae polyphenols). Fucophans are considered to be one of the main therapeutic components of brown algae\(^15\) and may constitute up to 25%–30% of the algal dry weight, depending on the specific seaweed species.\(^16\) Although fucophans are highly branched long chain polysaccharides, low levels of serum uptake of fucophans were observed after Undaria fucophan ingestion\(^17\) indicating that there is potential for clinical activity. Fucophans are a potent selenitogen blocker and has been used experimentally to prevent inflammatory damage after ischemic events.\(^18,19\)

Polyphenolic fractions derived from seaweed also have profound antioxidant activity\(^20\) which can also contribute to anti-inflammatory effects. Inhibition of oxalate damage to kidneys in animal models was attributed to the antioxidant qualities of Fucus-derived fucophan.\(^21\) Fucophans have been shown to inhibit phospholipase A\(_2\) \(^22\) an important enzyme in the inflammatory cascade inhibited by corticosteroids.

The toxicity of seaweed extracts has been investigated in both human and animal studies, although the source of the extracts has been different to the Fucus source used in this study. Previous human clinical studies using 3 g daily Undaria seaweed extracts with 75% fucophan content indicated no clinically observed toxicity.\(^23\) In a recent companion study on the coagulation effects of 3 g of the same Undaria extract, there was a significant change in clotting indices, but these remained within clinically normal parameters. Activated partial thromboplastin time increased from 28.41 to 34.01 s (n = 10; P = 0.01), thrombin time decreased from 18.62 to 17.55 s (n = 10, P = 0.04), and anti-thrombin-III increased from 113.5% to 117% (n = 10, P = 0.03).\(^24\) There were no toxicological changes observed in rats given up to 300 mg/kg orally of fucophan from Laminaria japonica. This dose is considerably higher than the 3000 mg per subject dose in the clinical studies. The absence of observations may be because of difference in the source species for the fucophan, or the absorption of the fucophan from the gut. Ancestral agents effects were observed at doses of 900 to 2,500 mg/kg, but no other signs of toxicity were observed.\(^25\) In a similar study involving fucophan extracted from Cladosiphon okamuranus, no significant toxicological changes were induced by fucophan at a dose of 600 mg/kg of body weight/day in Wistar rats. However, with concentrations at and above 1,200 mg/kg of body weight/day, clotting time was significantly prolonged.\(^26\) Overall, the dose levels used in this study (a total of either 100 mg and 1000 mg per day) would not be expected to produce changes outside of the clinically normal parameters, as they are many fold lower than the dose levels used in the animal studies, and a third of the dose used in the human clinical study. A polyphenol rich fraction of Fucus was also shown to lack acute toxic effects in rats after four weeks of oral dosing.\(^20\) The latter extract was somewhat different to the extract used in this study as it contained a concentrated polyphenol fraction.

The present study tested a seaweed extract nutrient complex containing a blend of extracts from three different species of brown algae plus nutrients zinc, manganese and B6, using an open label design at two doses to determine effects in OA. This was a pilot scale combined phase I and II study aimed at providing data on acute safety and efficacy.

**Material and methods**

**Research design**

This trial was a pilot scale open label dosing study, with two different doses randomised to participants, conducted over 12 weeks (84 days) in Lismore New South Wales (Australia) and was conducted in 2008. The study was approved by the Human Research Ethics Committee of Southern Cross University (Ethics approval number: ECN-07-36). The research was conducted in compliance with Good Clinical
Practices (GCP) and in accordance with the guidelines of the Australian National Health and Medical Research Council and the Declaration of Helsinki (as revised in 2004). The trial was registered with the Australian and New Zealand Clinical Trials Register (ACTRN12607000229471).

Participants
A convenience sample of healthy individuals aged between 18 and 65 years was recruited by email from staff and students at Southern Cross University, and from Lismore and surrounding areas through newspaper advertising, regional radio and television. All participants received a study information sheet outlining the study and signed an informed consent form agreeing to participate.

Participants were included if they had both X-ray and clinical evidence of osteoarthritis of the knees; if they had a baseline comprehensive osteoarthritis test (COAT) score between 3 and 7; were otherwise healthy (had no other acute nor chronic medical condition); and if they were willing to discontinue their current OA treatment for the duration of the study. Participants were excluded if they had a history of trauma associated with the affected joint; if they had rheumatoid arthritis or other inflammatory joint conditions (including gout); if they used corticosteroids (intra-articular or systemic) within four weeks prior to baseline and throughout the study; if they used anti-inflammatory agents or anti-arthritis complementary medicines three weeks prior to baseline and during the duration of the study; had liver function tests greater than three times the upper limit of normal at baseline; if they had a history of alcohol or substance abuse; were female participants who were lactating, pregnant or planning to become pregnant; if they had participated in another clinical trial in the last 30 days; if they were unwilling to have blood taken three times during the study; or if they were unwilling to comply with the study protocols.

Outcome measurements
The primary outcome measurement in this study were the safety measures and the average COAT score, a validated measurement instrument for the assessment of the symptoms of osteoarthritis. The COAT score is composed of four subscales: pain, stiffness, difficulty with physical activity and overall symptom severity. Secondary outcomes included the individual COAT sub-scales, TNF-alpha measurements, serum lipids and paracetamol usage over the 12 week study.

Baseline COAT measures were recorded and participants with visual analog scale (VAS) scores between 3 and 7 out of a possible 10 who agreed to wash out from their current osteoarthritis treatment were admitted to the study. Wash out commenced four weeks prior to the start of the trial and participants did not take their current osteoarthritis medications for the duration of the study. The participants were supplied with study diaries and asked to record their COAT scores and paracetamol use daily for four weeks. They were requested not to take narcotic analgesics and those containing codeine for seven days before the next clinic and until the end of the study. Study staff contacted the participants each week at the same time to ensure compliance and provide support.

COAT scores included joint pain, stiffness, difficulty with physical activities and overall symptom score. The participants completed a baseline COAT score under the supervision of the clinic staff by making a mark on a 10 cmVAS with a single vertical line to show the severity of each descriptor over the past twenty-four hours. The descriptors were none to extreme with a numerical score of 0 to 10. The scores were converted to a numerical grade through measurement with a ruler placed on the mark. Daily diaries were issued along with identical rulers (manufactured by Celco) and study staff contacted the participants each day at the same time to collect scores, ensure compliance and provide support. These instructions complied with the methods used with the validated tool. Participants were screened four weeks prior to the trial, they returned two weeks prior to ensure that scores remained between 3 and 7, and then returned at baseline. If the scores remained between 3 and 7 at baseline, the participant was randomised to a study medication.

Safety was assessed by actively monitoring adverse events. Subjects were questioned weekly about adverse events which were then recorded. Participants attended three clinics during which weight, blood pressure, pulse rate, and concomitant medication use was recorded and fasting blood samples collected at baseline, week 4, and week 12. Safety measurements undertaken by an independent accredited laboratory were full blood count, liver function tests and determination of urea, creatinine, electrolytes, cholesterol and triglyceride concentrations to assess toxicity to the hematopoietic, hepatic and renal systems; and assess the impact on fasting lipids. The body mass index (BMI) was calculated at the start and conclusion of the study.

Subjects returned all remaining capsules at each clinic visit and these were counted as a measure of compliance. The investigator maintained an inventory record of all capsules received and dispensed. It was assumed that capsules not returned were taken.
Participants were asked at the end of the study to evaluate the study medication by answering a question indicating their satisfaction, dissatisfaction, or neither satisfaction nor dissatisfaction with the study medication.

Study medication and dose
The study medication used Maritech® extract (Marinova Pty Ltd, Hobart, Australia). These are fucoidan-rich extracts of seaweeds (Maritech® extracts) which are manufactured using a proprietary aqueous process that produces fucoidan fractions. Nothing is added during the process. Insoluble matter and salts are removed as part of the process. They are ‘whole plant’ extracts which contain fucoidan and seaweed polyphenols (polyphloroglucinols) associated with the fucoidan molecule. In this study we used a blend of three Maritech® extracts from different species of brown seaweeds; Maritech® Fucus vesiculosus (85% w/w), Maritech® Macrocystis pyrifera (10% w/w) and Maritech® Laminaria japonica (5% w/w). In addition, Vitamin B6, zinc sulfate and manganese sulfate were included in the formulation, as detailed in Table 1. The study medication was manufactured as 100 mg and 250 mg capsules (Gel Caps) under the code of good manufacturing practice (GMP). The total fucoidan concentration in the 100 mg and 250 mg capsules was 75 mg and 187.5 mg respectively. Fucoidan content is assessed using a validated spectrophotometric method based on a modified Dubois method.28

Subjects were randomized to either a 100 mg or 1000 mg dose group using the research randomizer website (http://www.randomizer.org). Seven sets of two numbers per set, using a range of one to two, were generated. These numbers were used to assign the participants to either the 100 mg or 1000 mg dose. The 100 mg dose was delivered in one 100 mg gel capsule daily (taken in the morning) to five participants. The 1000 mg dose was delivered as four 250 mg capsules daily (two capsules taken twice daily) to seven participants. The capsules were self-administered orally by the participants after food.

Table 1 Contents of Maritech® capsules

<table>
<thead>
<tr>
<th>Component</th>
<th>100 mg</th>
<th>250 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maritech® Fucus vesiculosus</td>
<td>85 mg</td>
<td>212.5 mg</td>
</tr>
<tr>
<td>Maritech® Macrocystis pyrifera</td>
<td>10 mg</td>
<td>25 mg</td>
</tr>
<tr>
<td>Maritech® Laminaria japonica</td>
<td>5 mg</td>
<td>12.5 mg</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>57.5 mg</td>
<td>14.38 mg</td>
</tr>
<tr>
<td>Zinc sulphate monohydrate</td>
<td>25 mg</td>
<td>6.25 mg</td>
</tr>
<tr>
<td>Manganese sulphate</td>
<td>4 mg</td>
<td>1 mg</td>
</tr>
</tbody>
</table>

TNF alpha assays
After collection serum was stored at ~70 °C until analyzed. Serum tumor necrosis factor-α (TNF-α) was measured using a sandwich ELISA assay (Cayman Chemical Company, Denver, CO, USA). Thawed serum was supplemented with 5% mouse serum and 9 mM dithiothreitol in order to minimize nonspecific binding to the mouse anti-TNF-α. Serum was assayed without further dilution. TNF-α standards were prepared in TNF-α free human serum and similarly supplemented with mouse serum and dithiothreitol. Each sample was assayed in duplicate on two separate microwells (n = 4). Four samples had a single outlier removed. The standard curves for both plates had R²s of 0.99 over a concentration range of 0–250 pg/mL.

Statistical methods
A multilevel repeated measures analysis with auto-correlated time was conducted on the average COAT score and each of the four COAT subscales over the 85 measurement occasions from baseline (day 0) to completion (day 84) using SPSS (version 17.0; SPSS Inc, Chicago, IL, USA) with the Mixed procedure with a first order autoregressive (AR1) covariance modeled on the daily repeated measurements. The model presented fitted a two-piece linear response over time with a change point at day 21, and included the effects of treatment, 2-piece time, and 2-piece time by treatment interaction.

Results
Study participants
The study screened volunteers and subsequently enrolled thirteen people who met the inclusion/exclusion criteria and were comprised of six females (mean age ±SD 62.16 ±[9.04] years) and seven males (mean age ±SD 57.14 ±[9.20] years). One subject withdrew a week prior to the start of the baseline measure due to an acute respiratory tract infection. Eleven participants completed 12 weeks of the study. One participant completed 10 weeks of the study due to overseas travel for the final 2 weeks and their final blood measurement was taken at week 10. Data was analysed for these 12 participants. Five participants received the formulation in doses of 100 mg Maritech® extract and seven received 1000 mg Maritech® extract. There was 99.2% compliance at four weeks and 99.6% at twelve weeks with participants taking 100 mg daily and 97.4% at four weeks and 95.9% compliance at twelve weeks in individuals taking 1000 mg per day. Please see Figure 1 for a flow diagram of the trial. The study medication was well tolerated as all 12 participants were satisfied with the effects of taking the
study medication (compared with dissatisfied; or neither satisfied or dissatisfied).

The mean ages, and the baseline means for blood pressure, weight and pulse rate for the 100 mg and 1000 mg arms were not statistically different (data not presented).

COAT scores

Subjects taking either dose experienced reduction in COAT scores over the 12 weeks of the study. Means for the main outcome, the average of the COAT score subscales (average COAT), at baseline and on every seventh measurement occasion are provided for each treatment in Table 2 to summarize the response. A more complete description showing the daily mean average COAT scores is provided graphically in Figure 2 to which an empirical Loess curve was fitted to summarize the underlying response profiles. On observing Figure 2, two-piece linear functions with the break points at days 21 and 28 were tested in the statistical model for the Average COAT score, with the day 21 break point displaying superior fit in terms of the -2 log likelihood fit statistics (2007.9 at 21 days and 2010.7 at 28 days).

Table 2 reports the parameter estimates for the average COAT model and Figure 3 shows the estimated mean average COAT profiles by time by treatment. There was a highly significant treatment by time interaction effect (likelihood ratio test, chi-square = 15.155, df = 2, P = 0.0005), which was largely located after day 21 as indicated by the tests of the interaction parameters in Table 3. The parameter estimates are not reported for the pain, stiffness, physical difficulties and overall symptoms sub-scales but were similar to those obtained for the average COAT scale as would be expected from highly correlated variables. Table 4 reports the estimated means for each of the COAT scales at baseline and completion for both treatments, and the results of a one-tailed test of the hypothesis that the reduction from baseline to completion was significantly greater in the 1000 mg than the 100 mg treatment. This hypothesis was confirmed at P < 0.05 for the average COAT (0.043) scale, and the physical difficulties (0.010) and overall symptoms (0.044) sub-scales, but failed to reach significance for the...
pain (0.088) and stiffness (0.089) subscales. As shown in Table 4, mean average COAT reduced from 4.54 to 3.72 (18%) in the 100 mg treatment and from 4.81 to 2.32 (52%) in the 1000 mg treatment.

**Adverse events**

Six adverse events were noted. The first event, influenza occurred prior to the commencement of the trial and the participant withdrew. Two participants had hypertension, one baseline and one at week 4. Both participants had a history of hypertension. One participant had a chest infection at week 12, one had root canal work at week 12, and one participant had hyperacidity at week 12 with a history of gastric acidity at baseline. All events were considered to be unlikely to be related to the study medication.

**Blood safety measures**

There were no toxicity issues observed over the period of the study hemopoietic, hepatic and renal systems. Due to the small numbers of participants in this study it is not possible to consider gender differences. Table 5 presents the mean, standard deviation (SD) and sample size for each blood safety measure at baseline and completion in each treatment and a test of the significance of the change. There were no statistically significant changes in either treatment over time although there was a statistically significant but not clinically

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**Table 3 Average COAT scores: parameter estimates with their standard errors and tests of significance**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>t value</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept (100 mg)</td>
<td>4.342</td>
<td>0.705</td>
<td>6.442</td>
<td>13.699</td>
<td>0.000</td>
</tr>
<tr>
<td>Treatment (1000 mg)</td>
<td>0.263</td>
<td>0.923</td>
<td>0.285</td>
<td>13.698</td>
<td>0.780</td>
</tr>
<tr>
<td>Time 0–21 (days)</td>
<td>−0.036</td>
<td>0.018</td>
<td>−2.014</td>
<td>108.787</td>
<td>0.047</td>
</tr>
<tr>
<td>Time 21–84</td>
<td>−0.001</td>
<td>0.005</td>
<td>−0.227</td>
<td>87.490</td>
<td>0.821</td>
</tr>
<tr>
<td>Time 0–21 by treatment (1000 mg)</td>
<td>−0.027</td>
<td>0.023</td>
<td>−1.166</td>
<td>108.725</td>
<td>0.246</td>
</tr>
<tr>
<td>Time 21–84 by treatment (1000 mg)</td>
<td>−0.017</td>
<td>0.007</td>
<td>−2.539</td>
<td>87.311</td>
<td>0.013</td>
</tr>
<tr>
<td>Random: Subject level variance</td>
<td>2.078</td>
<td>0.949</td>
<td>2.190</td>
<td>87.311</td>
<td>0.029</td>
</tr>
<tr>
<td>Daily variance AR 1</td>
<td>0.739</td>
<td>0.059</td>
<td>12.528</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>rho AR 1</td>
<td>0.680</td>
<td>0.025</td>
<td>26.749</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: SE, standard error.
Figure 3 Average COAT score estimated mean by time by treatment.

Table 4 COAT scales: estimated baseline and completion means with 95% confidence intervals (CI) and tests of the difference in the reduction from baseline to completion between treatments

<table>
<thead>
<tr>
<th>COAT scale</th>
<th>Treatment</th>
<th>Time</th>
<th>Mean</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>100 mg</td>
<td>Baseline</td>
<td>4.542</td>
<td>3.027</td>
<td>6.057</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Completion</td>
<td>3.717</td>
<td>2.236</td>
<td>5.199</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>Baseline</td>
<td>4.805</td>
<td>3.524</td>
<td>6.086</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Completion</td>
<td>2.320</td>
<td>1.071</td>
<td>3.588</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Test</td>
<td></td>
<td>P = 0.043</td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>100 mg</td>
<td>Baseline</td>
<td>4.903</td>
<td>3.338</td>
<td>6.468</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Completion</td>
<td>3.827</td>
<td>2.300</td>
<td>5.355</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>Baseline</td>
<td>4.786</td>
<td>3.464</td>
<td>6.109</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Completion</td>
<td>2.122</td>
<td>0.086</td>
<td>3.409</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Test</td>
<td></td>
<td>P = 0.088</td>
<td></td>
</tr>
<tr>
<td>Stiffness</td>
<td>100 mg</td>
<td>Baseline</td>
<td>4.853</td>
<td>3.334</td>
<td>6.371</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Completion</td>
<td>3.605</td>
<td>2.123</td>
<td>5.088</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>Baseline</td>
<td>4.720</td>
<td>3.437</td>
<td>6.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Completion</td>
<td>2.339</td>
<td>1.090</td>
<td>3.588</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Test</td>
<td></td>
<td>P = 0.089</td>
<td></td>
</tr>
<tr>
<td>Physical difficulties</td>
<td>100 mg</td>
<td>Baseline</td>
<td>3.805</td>
<td>2.313</td>
<td>5.297</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Completion</td>
<td>3.667</td>
<td>2.209</td>
<td>5.125</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>Baseline</td>
<td>4.803</td>
<td>3.543</td>
<td>6.064</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Completion</td>
<td>2.401</td>
<td>1.173</td>
<td>3.629</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Test</td>
<td></td>
<td>P = 0.010</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>100 mg</td>
<td>Baseline</td>
<td>4.614</td>
<td>3.078</td>
<td>6.149</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Completion</td>
<td>3.769</td>
<td>2.267</td>
<td>5.271</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>Baseline</td>
<td>4.868</td>
<td>3.571</td>
<td>6.166</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Completion</td>
<td>2.413</td>
<td>1.147</td>
<td>3.678</td>
</tr>
</tbody>
</table>

Note: One-tailed test of the hypothesis that the reduction in mean scores from baseline to completion is greater in the 1000 mg than the 100 mg treatment.
Table 5. Blood safety measures: mean, standard deviation (SD) at baseline and completion 12 together with mean, SD, P-values for differences between baseline and completion by treatment.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 12</th>
<th>Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td><strong>Hematology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red cell count (x10^12/L)</td>
<td>100 mg</td>
<td>5</td>
<td>4.68</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>7</td>
<td>4.70</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>100 mg</td>
<td>5</td>
<td>150.40</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>7</td>
<td>146.71</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>100 mg</td>
<td>5</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>7</td>
<td>0.43</td>
</tr>
<tr>
<td>Mean corpuscular volume (fL)</td>
<td>100 mg</td>
<td>5</td>
<td>90.20</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>7</td>
<td>91.00</td>
</tr>
<tr>
<td>White cell count (x10^9/L)</td>
<td>100 mg</td>
<td>5</td>
<td>5.76</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>7</td>
<td>6.59</td>
</tr>
<tr>
<td>Lymphocytes (x10^9/L)</td>
<td>100 mg</td>
<td>5</td>
<td>1.96</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>7</td>
<td>2.04</td>
</tr>
<tr>
<td>Monocytes (x10^9/L)</td>
<td>100 mg</td>
<td>5</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>7</td>
<td>0.46</td>
</tr>
<tr>
<td>Neutrophils (x10^9/L)</td>
<td>100 mg</td>
<td>5</td>
<td>3.08</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>7</td>
<td>3.89</td>
</tr>
<tr>
<td>Basophils (x10^9/L)</td>
<td>100 mg</td>
<td>5</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>7</td>
<td>0.00</td>
</tr>
<tr>
<td>Eosinophils (x10^9/L)</td>
<td>100 mg</td>
<td>5</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
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<tr>
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</table>
significant increase in albumin over time for both treatments combined ($P = 0.034$).

**Paracetamol use**

Descriptive statistics for paracetamol use are reported in Table 6 together with Mann–Whitney tests comparing the treatments on use at baseline and total use over the duration of the trial. Weekly total paracetamol use (500 mg tablets) is plotted by treatment in Figure 4. None of subjects in the 100 mg treatment took any paracetamol on the first day of treatment while, of the seven subjects in the 1000 mg treatment, four took no paracetamol with the remaining three taking eight 500 mg tablets between them. The subjects in the 100 mg treatment took a total of 58 tablets (each 500 mg) over the duration of the trial with individual consumption ranging from 0 to 40 tablets, while the subjects in the 1000 mg treatment took a total of 152 tablets with individual consumption ranging from 0 to 62 tablets. Mann–Whitney comparisons of the two treatments showed no significant difference in consumption between the two groups either at baseline or over the duration of the trial.

**TNF-α**

Descriptive statistics for TNF-α are given in Table 7. There were no changes in TNF-alpha serum levels over time ($P = 0.170$), nor any differences between treatment groups ($P = 0.227$).

**Cholesterol and serum lipids**

Repeated measures analysis of variance was conducted on the baseline and 12 week values for serum lipids. Descriptive data is given in Table 8. One subject in the 100 mg group did not have a recordable low-density lipoprotein (LDL) in week 12. There were no differences found between the doses.

**Discussion**

This study investigated the effects on the symptoms of osteoarthritis using two doses of a complex containing a blend of Maritech® extracts from three brown algae species (*Fucus vesiculosus*, *Macrocytis pyrifera*, *Laminaria japonica*), plus vitamin B6, zinc and manganese in an open label design. There was a clear dose dependent effect on symptoms of osteoarthritis as assessed using the COAT index. The preparation was found to be safe at the doses used in the study population over 12 weeks.

After 12 weeks, the 100 mg dose reduced the average COAT score by 18% and the 1000 mg dose by 52%. The decrease in OA symptoms demonstrated by the 1000 mg dose is marked. Whilst there is no direct comparator in this study, results expected from nonsteroid anti-inflammatory drugs (NSAIDS) produce similar reductions in OA symptoms.6 Osteoarthritis studies often have large placebo responses and a limitation of this early investigation was a lack of a placebo arm. The significant difference between the two doses in a dose-dependent progression increases the likelihood that the 1000 mg dose is superior to a placebo.

A recent randomised controlled study on a mineral based supplement derived from seaweed undertaken over 12 weeks demonstrated changes in the symptoms of osteoarthritis when measured by WOMAC.8 Although derived from a seaweed, the mineral-based supplement was substantially different.
from the soluble fucoidan-rich preparations used in this study, and cannot be directly compared.

Individuals with OA show raised levels of TNF-α, and we hypothesized that the seaweed formulation would demonstrate a dose-dependent reduction over time as the fucoidans and polyphenols have been identified as anti-inflammatory agents. In this study, the TNF-α levels showed no change at either dosage; perhaps indicating that the effect observed is not mediated via a TNF-α-associated cascade. However, the study is not sufficiently powered to rule out a reduction in TNF-α, and further studies should include the measure.

Fucoidan is a major component of Maritech’s seaweed extracts. It is not a precursor to mammalian tissue formation, and is generally assumed to be impervious to mammalian enzyme breakdown. Fucoidans have profound selectin-blocking activity, and serum uptake of fucoidan has been demonstrated previously. It is possible that, in this clinical trial, selectin-blocking effects may reduce leukocyte accumulation in the osteoarthritis-affected areas, reducing the COAT scores via a generalized inhibition of inflammation. However paradoxically others have argued that selectin blockade may actually reduce pain relief. Further clinical trials and laboratory investigations are necessary to elucidate its mechanism of action.

The preparation was shown to be safe at the dosages consumed by the study population and any adverse effects were mild and self-limiting. There were no changes in the cholesterol, liver function, renal function, and hemopoietic function that were of any clinical significance during the course of the study.

The most significant limitation of this study was: being a pilot scale open label combined phase I and II trial it was subject to potential bias that would be reduced by the use of randomization to placebo or active with appropriate blinding. This study aimed to determine if this preparation had any potential in the treatment of the symptoms of osteoarthritis. The study achieved this aim and the significant dose responsiveness demonstrated provides evidence that these results are unlikely to be due to chance and deserve further exploration.

**Conclusion**

A seaweed extract nutrient complex when taken orally over twelve weeks significantly reduced the symptoms of osteoarthritis in a phase I and II open label study. The effect was highly dose-dependent with final reduction in COAT score of
Table 8 Cholesterol, HDL, LDL, ratio and triglycerides: means, SD, minima and maxima by treatment and measurement

<table>
<thead>
<tr>
<th>Cholesterol (mmol/L)</th>
<th>100 mg</th>
<th>1000 mg</th>
</tr>
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<tr>
<td>N</td>
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<td>SD</td>
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<tr>
<td>Baseline</td>
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<td>Ratio</td>
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<tr>
<td>Triglycerides</td>
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</tr>
<tr>
<td>Week 12</td>
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<tr>
<td>HDL</td>
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<td>1.18</td>
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<tr>
<td>Triglycerides</td>
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</table>

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.

18% and 52% respectively for the 100 mg and 1000 mg doses. The preparation was demonstrated to be safe to use over the study period at the doses tested in the study population. The observed pharmacological benefits now need to be demonstrated in a phase III randomized controlled trial.

Acknowledgments/disclosures

The study was sponsored by Marinova Pty Ltd under contract to Southern Cross University and performed independently by NatMed-Research. Dr Filton is employed by Marinova Pty Ltd. While she was involved in the study design, interpretation of results and preparation of the manuscript, she had no interaction with any study participant, nor was she involved in the day to day running or management of the clinical trial. Marinova Pty Ltd paid the article-processing charge associated with the publication of this paper. We thank Catherine Avila, Gareth Vanderhope and Airdre Grant at NatMed-Research who provided clinical research assistance; Dion Thompson who performed the TNF-α testing; the Northern Rivers Pathology Unit who undertook the safety measurements; and the participants who made it possible.

References
