GTC NUTRITION
SPECIALTY INGREDIENTS

February 23, 2007

Program Manager, USDA/AMS/TM/NOP
Room 4008-So., Ag Stop 0268
1400 Independence Ave., SW.
Washington, DC 20250
Phone: 202-720-3532
Fax: 202-205-7808

Dear Program Manager:

Please find enclosed duplicate copies of GTC Nutrition's petition to have Aquamin™ F, seaweed derived calcium included on the National List of Allowed Substances in Organic Production. If you have any questions or need additional information please contact me directly.

Sincerely,

Luke R. Kazmierski
Quality Assurance and Regulatory Affairs Specialist
GTC Nutrition
Phone: 303-216-2489
E-mail: lkazmierski@gtcnutrition.com

GTC NUTRITION

SPECIALTY INGREDIENTS

Aquamin™ F

Petition for Inclusion on the National List of Allowed Substances in Organic Production

CBI - Deleted

1	Petition for Inclusion on the National List of Allowed Substances
2	Appendix 1 Process Flow Diagram – CBI Deleted
3	Appendix 2 GRAS Letter
4	Appendix 3 Certificate of Health – Ireland
5	Appendix 4 Organic Trust Limited Certificate
6	Appendix 5 List of International Regulatory Status
7	Appendix 6 MSDS
8	Appendix 7 Safety/Studies

Category 1. Adverse impacts on humans or the environment? Substance: Aquamin F

Question	Yes	No	N/A[1]	Documentation (TAP; petition; regulatory agency; other)
1. Are there adverse effects on environment from manufacture, use, or disposal? [§205.600 b.2]		√		**There is no toxicity or environmental persistence as this is a naturally occurring calcium source produced from mineralized seaweed found off the Irish coast. Please see attached MSDS (See Appendix 6).**
2. Is there environmental contamination during manufacture, use, misuse, or disposal? [§6518 m.3]		√		**There is no toxicity or environmental persistence as this is a naturally occurring calcium source produced from mineralized seaweed found off the Irish coast. Please see attached MSDS (See Appendix 6).**
3. Is the substance harmful to the environment? [§6517c(1)(A)(i);6517(c)(2)(A)i]		√		**There is no toxicity or environmental persistence as this is a naturally occurring calcium source produced from mineralized seaweed found off the Irish coast. Please see attached MSDS (See Appendix 6).**
4. Does the substance contain List 1, 2, or 3 inerts? [§6517 c (1)(B)(ii); 205.601(m)2]		√		**There is no toxicity or environmental persistence as this is a naturally occurring calcium source produced from mineralized seaweed found off the Irish coast. Please see attached MSDS (See Appendix 6).**
5. Is there potential for detrimental chemical interaction with other materials used? [§6518 m.1]		√		**This product is inert.**
6. Are there adverse biological and chemical interactions in agro-ecosystem? [§6518 m.5]		√		**This substance is intended as an ingredient in food products and exists as a naturally occurring calcium source produced from mineralized seaweed found off the Irish coast. The substance is GRAS recognized (See Appendix 2). Please see attached MSDS (See Appendix 6).**
7. Are there detrimental physiological effects on soil organisms, crops, or livestock? [§6518 m.5]		√		**This substance is intended as an ingredient in food products and exists as a naturally occurring calcium source produced from mineralized seaweed found off the Irish coast. The substance is GRAS recognized (See Appendix 2). Please see attached MSDS (See Appendix 6).**
8. Is there a toxic or other adverse action of the material or its breakdown products? [§6518 m.2]		√		**There is no toxicity or environmental persistence as this is a naturally occurring calcium source produced from mineralized seaweed found off the Irish coast. Please see attached MSDS (See Appendix 6).**
9. Is there undesirable persistence or concentration of the material or breakdown products in environment?[§6518 m.2]		√		**There is no toxicity or environmental persistence as this is a naturally occurring calcium source produced from mineralized seaweed found off the Irish coast. Please see attached MSDS (See Appendix 6).**
10. Is there any harmful effect on human health? [§6517 c (1)(A)(i) ; 6517 c(2)(A)i; §6518 m.4]		√		**There is no toxicity or environmental persistence as this is a naturally occurring calcium source produced from mineralized seaweed found off the Irish coast. Please see attached MSDS (See Appendix 6).**
11. Is there an adverse effect on human health as defined by applicable Federal regulations? [205.600 b.3]		√		**This product is a naturally occurring calcium source produced from mineralized seaweed found off the Irish coast which is GRAS recognized (See Appendix 2).**
12. Is the substance GRAS when used according to FDA's good manufacturing practices? [§205.600 b.5]	√			**The GRAS Notice Number is GRN000028 (See Appendix 2).**
13. Does the substance contain residues of heavy metals or other contaminants in excess of FDA tolerances? [§205.600 b.5]		√		**This product is GRAS recognized and does not exceed FDA tolerances (See Appendix 2).**

[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.

Category 2. Is the Substance Essential for Organic Production? Substance: Aquamin F

Question	Yes	No	N/A[1]	Documentation (TAP; petition; regulatory agency; other)
1. Is the substance formulated or manufactured by a chemical process? [6502 (21)]		√		**This is a naturally occurring unique red algae product known as *Lithothamnium Corallioides* found off the Irish coast. Please see attached flow diagram (See Appendix 1).**
2. Is the substance formulated or manufactured by a process that chemically changes a substance extracted from naturally occurring plant, animal, or mineral, sources? [6502 (21)]		√		**This is a naturally occurring unique red algae product known as *Lithothamnium Corallioides* found off the Irish coast. Please see attached flow diagram (See Appendix 1).**
3. Is the substance created by naturally occurring biological processes? [6502 (21)]	√			***Lithothamnium Corallioides* is naturally occurring red algae and therefore grows sublittorally for a number of years prior to becoming calcified and falling to the seabed. Please see attached flow diagram (See Appendix 1).**
4. Is there a natural source of the substance? [§205.600 b.1]	√			**The product exists in nature as red algae known as *Lithothamnium Corallioides.***
5. Is there an organic substitute? [§205.600 b.1]		√		**This is a naturally occurring unique red algae product known as *Lithothamnium Corallioides* found off the Irish coast.**
6. Is the substance essential for handling of organically produced agricultural products? [§205.600 b.6]	√			**Please see attached an organic certificate from the Organic Trust in Ireland (See Appendix 4).**
7. Is there a wholly natural substitute product? [§6517 c (1)(A)(ii)]		√		**This is a naturally occurring unique red algae product known as *Lithothamnium Corallioides* found off the Irish coast.**
8. Is the substance used in handling, not synthetic, but not organically produced? [§6517 c (1)(B)(iii)]		√		**This is a naturally occurring unique red algae product known as *Lithothamnium Corallioides* found off the Irish coast.**
9. Is there any alternative substances? [§6518 m.6]		√		**This is a naturally occurring unique red algae product known as *Lithothamnium Corallioides* found off the Irish coast.**
10. Is there another practice that would make the substance unnecessary? [§6518 m.6]		√		**This is a naturally occurring unique red algae product known as *Lithothamnium Corallioides* found off the Irish coast.**

[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.

Category 3. Is the substance compatible with organic production practices? Substance: Aquamin F

Question	Yes	No	N/A[1]	Documentation (TAP; petition; regulatory agency; other)
1. Is the substance compatible with organic handling? [§205.600 b.2]	√			**This substance is intended as an ingredient in food products. Studies have shown the numerous health benefits when consuming this product (See Appendix 7).**
2. Is the substance consistent with organic farming and handling? [§6517 c (1)(A)(iii); 6517 c (2)(A)(ii)]	√			**This substance is intended as an ingredient in food products. Studies have shown the numerous health benefits when consuming this product (See Appendix 7).**
3. Is the substance compatible with a system of sustainable agriculture? [§6518 m.7]	√			**This is a naturally occurring product found off the Irish coast.**
4. Is the nutritional quality of the food maintained with the substance? [§205.600 b.3]	√			**This substance improves the nutritional quality of foods in which it is added.**
5. Is the primary use as a preservative? [§205.600 b.4]		√		**The substance is intended as an ingredient in food with no preservative effect.**
6. Is the primary use to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law, e.g., vitamin D in milk)? [205.600 b.4]		√		**This substance which is intended as an ingredient in food, is a natural, sea derived calcium and multi mineral source used for fortification and enrichment of the nutritional value of food products in which it is added.**
7. Is the substance used in production, and does it contain an active synthetic ingredient in the following categories: a. copper and sulfur compounds;			√	**This substance which is intended as an ingredient in food, is a natural, sea derived calcium and multi mineral source used for fortification and enrichment of the nutritional value of food products in which it is added.**
b. toxins derived from bacteria;			√	**N/A**
c. pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals?			√	**N/A**
d. livestock parasiticides and medicines?			√	**N/A**
e. production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleaners?			√	**N/A**

[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.

NOSB RECOMMENDED DECISION

Form NOPLIST2. Full Board Transmittal to NOP

For NOSB Meeting: _____ | Substance: _____

A. Evaluation Criteria (Documentation attached; committee recommendation attached)

	Criteria Satisfied?
1. Impact on humans and environment	Yes ☐ No ☐ (see B below)
2. Availability criteria	Yes ☐ No ☐ (see B below)
3. Compatibility & consistency	Yes ☐ No ☐ (see B below)

B. Substance fails criteria?

Criteria category: _____

Comments:

C. Proposed Annotation: _____

_____

Basis for annotation:

To meet criteria above: ____ Criteria: _____

Other regulatory criteria: ____ Citation:_____

D. Final Board Action & Vote: Motion by: _____ Second:_____

Vote:

Yes: _____

No: _____

Abstain: _____

Agricultural		Nonagricultural		Crops	
Synthetic		Not synthetic		Livestock	
Allowed[1]		Prohibited[2]		Handling	
No restriction		Deferred4		Rejected[3]	

1—substance voted to be added as "allowed" on National List

Annotation: _____

_____

2—substance to be added to "prohibited" paragraph of National List

Describe why a prohibited substance:_____

_____

3—substance was rejected by vote for amending National List

Describe why material was rejected:_____

_____

4-substance was recommended to be deferred

Describe why deferred; if any follow-up is needed. If follow-up needed, who conducts follow-up._____

_____

E. Approved by NOSB Chair to transmit to NOP:

_____ _____

Dave Carter, NOSB Chair Date

F. NOP Action: Include in FR to amend National List: ☐

Return to NOSB ☐ Reason: _____

_____ _____

Richard H. Mathews, Program Manager Date

NOSB COMMITTEE RECOMMENDATION

Form NOPLIST1. Committee Transmittal to NOSB

For NOSB Meeting: _____ | Substance: _____

Committee: Crops ☐ Livestock ☐ Handling ☐

A. Evaluation Criteria (Documentation attached; committee recommendation attached)

	Criteria Satisfied?
4. Impact on humans and environment	Yes ☐ No ☐ (see B below)
5. Availability criteria	Yes ☐ No ☐ (see B below)
6. Compatibility & consistency	Yes ☐ No ☐ (see B below)

B. Substance fails criteria?

Criteria category: _____

Comments:

C. Proposed Annotation: _____

_____

Basis for annotation:

To meet criteria above: ____ Criteria: _____

Other regulatory criteria: ____ Citation: _____

D. Recommended Committee Action & Vote: Motion by: _____

Seconded: _____

Vote:

Yes: _____

No: _____

Abstain: _____

Agricultural		Nonagricultural		Crops	
Synthetic		Not synthetic		Livestock	
Allowed[1]		Prohibited[2]		Handling	
No restriction		Deferred4		Rejected[3]	

1—substance voted to be added as "allowed" on National List

Annotation: _____

_____

2—substance to be added to "prohibited" paragraph of National List

Describe why a prohibited substance: _____

_____

3—substance was rejected by vote for amending National List

Describe why material was rejected: _____

_____

4-substance was recommended to be deferred

Describe why deferred; if follow-up is needed. If follow-up needed, who will follow up _____

_____

E. Approved by Committee Chair to transmit to NOSB:

_____ _____

Committee Chair Date

Received
03/02/07

Item A:

Category: §205.605(a) Non-agricultural (nonorganic) nonsynthetic substances allowed in or on processed products labelled as "organic" or "made with organic (specified ingredients)."

Item B:

1. **Common name of substance:**

Aquamin F, seaweed derived calcium *(Lithothamnium Corallioides)*

2. **Manufacturer's information:**

Marigot Limited,
Strand Farm,
Currabinny,
Carrigaline,
Co. Cork,
Ireland

Marigot Limited Asia-Pacific Office,
52 Turton Avenue,
Clemton Park,
NSW 2206,
Sydney,
Australia

3. **Intended or current use:**

Ingredient in food products

4. **Handling activity:**

Aquamin F is normally added to other dry ingredients.

5. Source and manufacturing procedures:

The raw material is a naturally occurring calcium source produced from mineralized seaweed found off Atlantic Waters of the Irish coast. Aquamin F is then produced by washing and milling the seaweed derived calcium. Please see attached flow chart (see Appendix 1).

6. Summary of previous regulatory reviews:

GRAS letter (See Appendix 2)

EU Health Cert (See Appendix 3)

7. Information regarding regulatory registrations:

List of international regulatory/claim status (See Appendix 5)

8. CAS number:

None

9. Chemical properties and mode of action

A) The substance, Lithothanium consists mainly of mineral substances (95-99.5%). The main constituents are calcium carbonate (32 to 36%) and magnesium carbonate (2.7 to 3.3%) as well as another 70 trace minerals.

B) There is no toxicity or environmental persistence as this is a naturally occurring calcium source produced from mineralized seaweed found off the Irish coast.

C) This type of product has no significant effect on the human environment due to it being a naturally occurring calcified seaweed found off the Irish coast.

D) Effects on human health are attached (See Appendix 7). Generally the product is used for the improvement of human bone health. It has been demonstrated to positively influence bone resorption markers and bone density, thus stimulating an overall improvement in bone health.

demonstrated to positively influence bone resorption markers and bone density, thus stimulating an overall improvement in bone health.

10. Safety information:

MSDS attached (See Appendix 6)

11. Research reviews provided:

The research reviews provided pertain to health benefits (See Appendix 7).

12. Petition justification statement:

The product falls under the category §205.605(a) Non-agricultural (nonorganic) nonsynthetic substances allowed in or on processed products labelled as "organic" or "made with organic (specified ingredients)." There are currently no organic equivalents of the product available. The product is not synthetic, it is a naturally occurring calcium source produced from mineralized seaweed found off the Irish coast. Therefore Aquamin F should be included on the National List, as it provides a valuable source of highly bioavailable, lactose free, naturally occurring calcium and 73 other trace minerals. Aquamin F is easily incorporated into a wide range of foods, snacks, beverages and dietetic foods and leads to interesting documented health benefits at low inclusion levels.

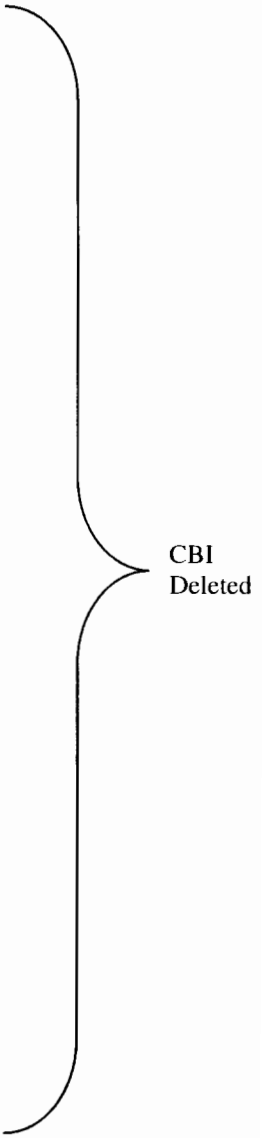
13. Commercial confidential information statement:

The process flow chart for the manufacturing of Aquamin F is considered confidential business information (CBI). This diagram is located in Appendix 1.

1

CBI Deleted

AQUAMIN F PROCESS FLOW DIAGRAM



JUN 05

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
Washington, DC 20204

April 21, 2000

Mr. Michael Ryan
Marigot Ltd.
Strand Farm, Currabinny
Carrigaline, Co. Cork
IRELAND

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Re: GRAS Notice No. GRN 000028

Dear Mr. Ryan:

The Food and Drug Administration (FDA) is responding to the notice, dated July 9, 1999, that you submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS)). FDA received the notice on July 28, 1999 and designated it as GRAS Notice No. GRN 000028.

The subject of the notice is a product that you call "calcified seaweed" or "Maerl." The notice informs FDA of the view of Marigot, Limited (Marigot) that "calcified seaweed" derived from *Phymatolithon calcareum* or *Lithothamnium corrallioides* is GRAS, through scientific procedures, for use as a source of dietary calcium for food enrichment and fortification purposes. The typical level of use of "calcified seaweed" is less than 2 per cent and the maximum level of use is 4 per cent.

According to your notice, "calcified seaweed" is a naturally occurring photosynthetic product, of marine origin, that accumulates in submarine banks or deposits over time. Although its composition can vary depending on the point of harvest, season, or depth of the deposit, it typically contains 84.2 per cent calcium carbonate and 11.4 per cent magnesium carbonate. The balance is moisture (typically 0.5 to 2.0 per cent) and trace elements. Given this composition, it is the view of the Office of Premarket Approval that the term "calcified seaweed" does not adequately describe the substance that is the subject of your notice because it implies, inaccurately, that the characterizing property of the substance is "seaweed" rather than "calcium." Therefore, for the purpose of this letter, we are using the term "seaweed-derived calcium" to describe the subject of your notice. We have provided a copy of your notice to Mr. John Foret, Office of Nutritional Products, Labeling, and Dietary Supplements, Division of Compliance and Enforcement, Center for Food Safety and Applied Nutrition, HFS-156, 200 C Street S.W., Washington, DC 20204. We recommend that you contact Mr. Foret to discuss the common or usual name that would be used to identify seaweed-derived calcium in the ingredient statement of food products that contain this ingredient and would be marketed in the U.S. You can reach

Mr. Foret by telephone at (202)205-5229, by telefax at (202)205-4594, or by electronic mail at JForet@cfsan.fda.gov.

In your notice, you provide a typical range for the levels of the major components of seaweed-derived calcium (i.e., calcium, 31 to 34 percent; magnesium, 2.8 to 3.3 percent; and moisture, less than 5 percent). You also provide a typical range for the levels in the final product of trace elements such as lead (less than 0.9 parts per million (ppm), iodine, boron, and selenium. In the section of your notice entitled "Specifications," you note that seaweed-derived calcium is a natural photosynthetic product of marine origin that is subject to variation, and that the typical values provided are subject to some seasonal variation.

The major component of seaweed-derived calcium, calcium carbonate, is affirmed as GRAS for use in food with no limitation other than current good manufacturing practice (21 CFR 184.1191). Seaweed-derived calcium is processed using a certified ISO 9000 procedure for food grade products. The seaweed-derived calcium is sterilized and deodorized in a heated solution of hydrogen peroxide for 90-120 minutes, depending on the rate of decomposition of the hydrogen peroxide. It is then milled and bagged under sterile conditions.

You intend to use seaweed-derived calcium as a source of calcium in food products such as biscuits, pasta, hard candy, tomato juice, soya milk, soya desserts and yoghurt, dairy yoghurt, dairy desserts, dietetic foods, and soups, at a typical level of less than 2 per cent. According to your notice, the use of "seaweed-derived calcium" is limited by its effects on palatability and texture, with an upper limit of 4 per cent.

Based on the information provided by Marigot, as well as other information available to FDA, the agency has no questions at this time regarding the conclusion of Marigot that seaweed-derived calcium is GRAS under the proposed conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of seaweed-derived calcium. As always, it is your continuing responsibility to ensure that food ingredients that you market are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter, as well as a copy of the information in your notice that conforms to the information in proposed 21 CFR 170.36(c)(1), is available for public review and copying on the Office of Premarket Approval's homepage on the World Wide Web.

Sincerely,
/s/
Alan M. Rulis, Ph.D.
Director
Office of Premarket Approval
Center for Food Safety and Applied Nutrition

Food Safety
AUTHORITY OF IRELAND

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21 August 2008

CERTIFICATE OF HEALTH

To Whom it may Concern

The Food Safety Authority of Ireland hereby certifies that **"Aquamin F" – Natural Calcium Source** produced by the Marigot Ltd., Cork, Ireland, is manufactured in accordance with the Food Hygiene (Ireland) Regulations 2006 (S.I. No. 369 of 2006) and the Food Safety Authority of Ireland Act, 1998.

Judith Giles
Signed on behalf of the
Food Safety Authority of Ireland

Food Safety
AUTHORITY OF IRELAND

Official Stamp of the
Food Safety Authority of Ireland

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ORGANIC TRUST LIMITED
CERTIFIED PRODUCTS SCHEME

This is to certify that the Organic Trust Symbol under the Certified Products Scheme has been awarded to

*AquaMin * AquaCal * SeaCal * Acid But*
*BioFilter Media * Dri-Li * Lithothamnion Powder*

The OT Certified Products Scheme covers approved products acceptable for use in organic systems- such products lie outside the legislative scope of (EEC) Regulation 2092/91 as amended

Certification for above product has been granted to

Marigot
T/A Celtic Sea Minerals
(Michael Ryan, Denis O'Neill & AP Stanway)

at

Strand Farm, Currabinny, Carrigaline, Co Cork
Celtic Sea Minerals, Dinish Island, Castletownbere, Co Cork
Microferm Ltd, Spring Lane North, Malvern Link, Worcestershire WR14 1BU

Symbol No. CP300 ***Valid until: 31.12.2007***

Signed: ***Date: 01.01.2007***

National Co-ordinator of the Organic Trust Ltd,
Vernon House, 2 Vernon Avenue, Clontarf, Dublin 3.
Telephone/Fax: 01 8530271. Email: organic@iol.ie

The Organic Trust Limited is an EU Approved Organic Certification Body. EN45011 compliant.

Regulatory Status/Claims 3/2006

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Argentina

Food Ingredients – Approved

Australia/New Zealand

Whole Food, Seaweed Calcium – Approved

Austria

Food Ingredients – Approved

Belgium

Food Ingredients – Approved

Brazil

Food Ingredients – Approved

Canada

Food Ingredients – Approved

Chile

Food Ingredients – Approved

Colombia

Food Ingredients – Approved

Czech Republic

Food Ingredients – Approved

Denmark

Food Ingredients – Approved

Finland

Food Ingredients – Approved

France

Food Ingredients – Approved

Germany

Food Ingredients – Approved

Italy

Food Ingredients – Approved

Japan

Whole Food, Seaweed Calcium – Approved

Mexico

Food Ingredients – Approved

Norway

Food Ingredients – Approved

Poland

Food Ingredients – Approved

Portugal

Food Ingredients – Approved

Slovenia

Food Ingredients – Approved

South Africa

Food Ingredients – Approved

South Korea

Whole Food, Seaweed Calcium – Approved

Spain

Food Ingredients – Approved

Sweden

Food Ingredients – Approved

Switzerland

Food Ingredients – Approved

Taiwan

Whole Food, Seaweed Calcium – Approved

Thailand

Whole Food, Seaweed Calcium – Approved

The Netherlands

Food Ingredients – Approved

The Philippines

Whole Food, Seaweed Calcium – Approved

Turkey

Food Ingredients – Approved

United Kingdom

Food Ingredients – Approved

United States of America

Food Ingredients – Approved

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MARIGOT
IRELAND LTD

MSDS 1 REVISION 6	ISSUED BY	October 2004
To conform with ISO 11014 -1	R&D, MARIGOT Ltd.	Page 1 of 4

1. Product and Company Identification

BOTANICAL NAME: *Lithothamnium corallioides/Lithothamnium calcareum*

SYNONYMS/ TRADE NAMES: Aquacal®, Aquamin®F, Aquamin®TG

SUPPLIED BY:

Marigot Ltd.,
Strand Farm,
Currabinny,
Carrigaline,
Co. Cork.
Republic of Ireland.

Tel: +353 21 4378727
Fax: +353 21 4378588

2. Composition Information on Ingredients

Aquacal®, Aquamin®F, Aquamin®TG comprises a mineralised seaweed extract (sp. *Lithothamnium*) with the principal mineral constituent being Calcium Carbonate.

3. Hazards Identification

Prolonged exposure to powdered products may cause mechanical irritation to eyes and lungs.

4. First Aid Measures

Exposure Route	Symptom	Treatment
Inhalation	Mild Irritation	Remove from exposure. If symptoms continue seek medical attention.
Eye Contact	Irritation	Rinse thoroughly with water for at least 15 minutes. If irritation persists seek medical attention

Exposure Route	Symptom	Treatment
Ingestion	Mild Irritation	Wash out mouth with water. If patient feels unwell seek medical attention.

5. Fire Fighting Procedures.

Does not support combustion.

Suitable Fire Extinguishing Media	Standard media such as powder, foam or water are suitable.
Hazardous Combustion Products.	N/A

6. Accidental Release Measures

Safety Precautions	Use goggles, dust mask and gloves as per GMP, particularly in instances of high dust concentration.
Clean up procedure	Shovel, sweep, vacuum.

7. Handling & Storage

Appropriate measures should be taken to avoid formation of dust. Local exhaust recommended if handling large quantities. Store in a cool, dry area.

8. Exposure Controls/Personal Protection.

Personal Protective Equipment:

Respiratory Protection:	Approved dust mask in high dust concentrations.
Hand:	Gloves
Eye:	Goggles
Skin:	Overalls

9. Physical & Chemical Properties

Appearance

Aquacal®	Cream/off-white, fine, odourless powder.
Aquamin®F	Cream/off-white, fine, odourless powder
Aquamin®TG	Cream/off-white, free flowing odourless granules.

Decomposition Temperature	>850°C
Flashpoint	Not applicable.
Flammability	Does not support combustion
Solubility	Insoluble in water, alcohol and most organic solvents.

10. Stability & Reactivity

Stability	Stable under normal conditions.
Potentially hazardous reactions	Reacts with acids liberating Carbon Dioxide
Conditions to avoid	N/A
Materials to avoid	Acids.

11. Toxicological Information

Local Effects	Fine powders may irritate the upper respiratory tract, prolonged skin contact may cause irritation to the eyes. Unlikely to be hazardous if swallowed.

12. Ecological Information

Environmental Effects	Insoluble in water and can easily be separated from aqueous systems by sedimentation or filtration.
Aquatic Toxicity	N/A

13. Disposal Considerations

Landfill	Comply with local authority regulations.

14. Transport Information

Not regulated as this product is not classified as dangerous goods.

15. Regulatory Information

No risks or hazards. Not regulated. Conform with local authority regulations.

16. Other Information

These products are intended for use as food ingredients and in dietary supplements.

The information provided in the MSDS is correct to the best of our knowledge, information and belief at the date of its publication and is in our opinion consistent with the state of general scientific and technical knowledge at that date. MARIGOT LTD. cannot accept liability for any loss, injury or damage, which may result from its use.

In compiling this MSDS we have taken into account all proper applications of the material of which we are aware. It is the responsibility of any intermediate supplier to ensure that the information contained in this MSDS is passed to the ultimate user.

VII. Meta-Analysis of Calcium Supplementation for the Prevention of Postmenopausal Osteoporosis

BEVERLEY SHEA, GEORGE WELLS, ANN CRANNEY, NICOLE ZYTARUK, VIVIAN ROBINSON, LAUREN GRIFFITH, ZULMA ORTIZ, JOAN PETERSON, JONATHAN ADACHI, PETER TUGWELL, GORDON GUYATT, THE OSTEOPOROSIS METHODOLOGY GROUP, AND THE OSTEOPOROSIS RESEARCH ADVISORY GROUP

A. Abstract

Objective: To summarize controlled trials examining the effect of calcium on bone density and fractures in postmenopausal women.

Data Source: We searched MEDLINE and EMBASE up to 1998 and the Cochrane Controlled Register up to 2000, and we examined citations of relevant articles and proceedings of international meetings. We contacted osteoporosis investigators to identify additional studies, and primary authors for unpublished data.

Study Selection: We included 15 trials (1806 patients) that randomized postmenopausal women to calcium supplementation or usual calcium intake in the diet and reported bone mineral density of the total body, vertebral spine, hip, or forearm, or recorded the number of fractures, and followed patients for at least 1 yr.

Data Extraction: For each trial, three independent reviewers assessed the methodological quality and extracted data.

Data Synthesis: We found calcium to be more effective than placebo in reducing rates of bone loss after two or more years of treatment. The pooled difference in percentage change from baseline was 2.05% [95% confidence interval (CI) 0.24–3.86] for total body bone density, 1.66% (95% CI 0.92–2.39) for the lumbar spine, 1.64% (95% CI 0.70–2.57) for the hip, and 1.91% (95% CI 0.33–3.50) for the distal radius. The relative risk (RR) of fractures of the vertebrae was 0.77 with a wide CI (95% CI 0.54–1.09); the RR for nonvertebral fractures was 0.86 (95% CI 0.43–1.72).

Conclusions: Calcium supplementation alone has a small positive effect on bone density. The data show a trend toward reduction in vertebral fractures, but do not meaningfully address the possible effect of calcium on reducing the incidence of nonvertebral fractures.

B. Introduction

OF ALL THE available preventive strategies for osteoporotic fractures, calcium is the simplest and least expensive. An essential nutrient with minimal toxicity, calcium supplementation is nevertheless not without controversy (1, 2). The Food and Drug Administration in the United States has permitted a bone health claim for calcium rich foods, and the NIH in its Consensus Development Process approved a statement that high calcium intake reduces the risk of osteoporosis.

Cumming *et al.* (3) reviewed both observational and controlled clinical trials relating calcium intake to fracture incidence. Observational studies often provide biased estimates, and the authors did not find conclusive evidence of benefit from the controlled trials alone. Furthermore, they did not examine the effect of calcium supplementation on bone mineral density (3). Mackerras and Lumley (4) conducted a meta-analysis of randomized controlled trials (RCTs) examining the effect of increasing calcium ingestion on bone density in women, but their analysis omitted 4 of the 15 available studies, failed to contact authors to obtain missing data and clarify data report accuracy, and did not address the effect on fractures. We have therefore conducted a systematic review to quantify the effect of calcium supplementation on postmenopausal bone loss and fractures.

This section is the seventh in our series presenting RCT evidence regarding major antiosteoporotic therapy. In *Section I*, we presented the rationale for the series and described in detail the methods common to each systematic review. In this analysis, we will briefly summarize our methods and consider the effect of calcium supplementation alone. We deal with studies that examined the effects of calcium and vitamin D given together in the next section.

C. Methods

1. Inclusion criteria. We developed and published an *a priori* protocol according to the methods recommended by the Cochrane Collaboration (5). Studies satisfied the following inclusion criteria, as indicated in *Section I*, as well as the following: 1) RCTs of calcium supplementation in women older than 45 yr with absence of menses for a minimum of 6 months; 2) treatment with doses of calcium at least 400 mg/d. We also included RCTs in which both active and control groups received a maintenance dose of vitamin D, providing the loading dose was no more than 300,000 IU, and the maintenance dose was no more than 400 IU/d (6, 7).

2. Study search and selection. To identify RCTs of calcium supplementation, we evaluated MEDLINE and EMBASE from January 1966 to April 1998 including Current Contents of the 6 months before April 1998, and the Cochrane Controlled Trials Register up to 2000 (8, 9). We also conducted hand searches of bibliographic reference. We asked content experts to identify published or unpublished relevant RCTs.

Abbreviations: CI, Confidence interval; RCT, randomized controlled trial; RR, relative risk.

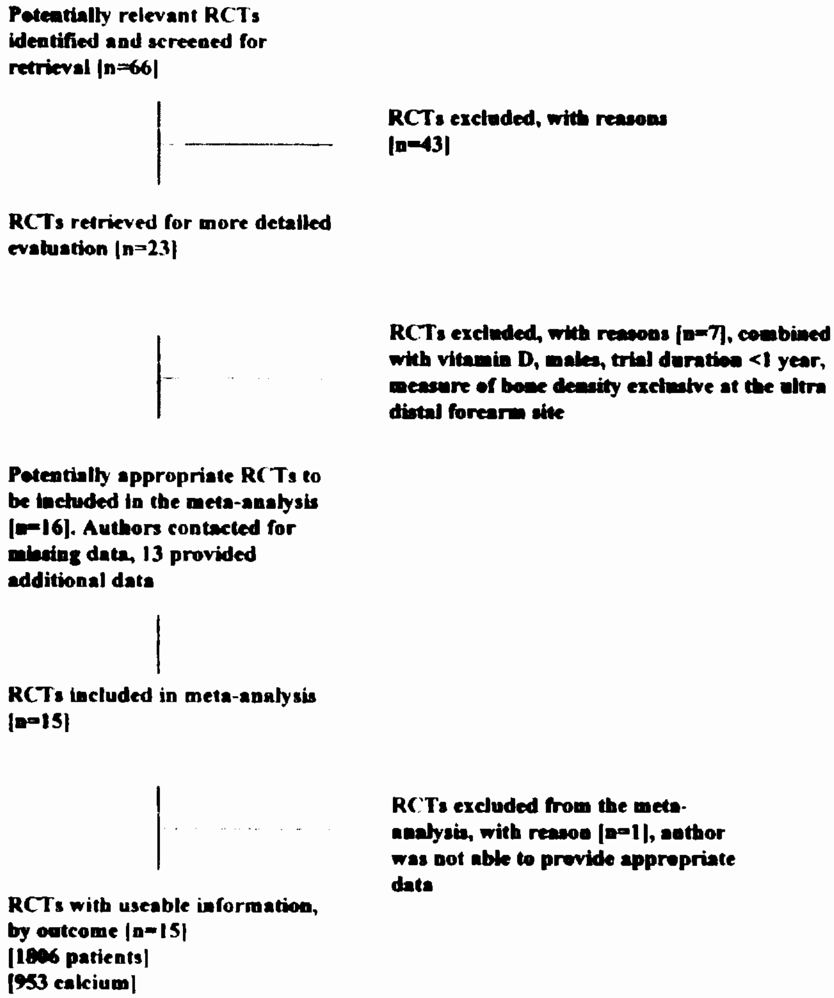


FIG. 1. Results of search for eligible studies.

we had overlooked. Two reviewers (J.P., B.S.) examined each title generated from the search and identified potentially eligible articles for which we obtained the abstracts. For abstracts consistent with study eligibility, we obtained the full article text.

3. *Methodological quality.* Three reviewers (J.P., N.Z., B.S.) rated the methodological quality of each eligible study with respect to whether patients, caregivers, and those measuring outcome are blind to allocation, and the extent of loss to follow-up.

4. *Reliability of judgements.* We used more than one reviewer in the selection of studies, the assessment of methodological quality, and the extraction of data. For all aspects of the review in which raters made duplicate judgements, they resolved disagreements by consensus. The interobserver agreement measured for the quality assessment with κ (10) for blind to allocation 0.85, and for follow-up was 0.49.

5. *A priori hypotheses regarding heterogeneity.* To explore reasons for large differences in results between studies (heterogeneity) we developed *a priori* hypotheses relating to the methodological quality of the study, the study population, and the dose and type of calcium administered. Specifically, we compared results in RCTs grouped in the following ways: 1) different methodological quality (randomization concealed or unconcealed; blinded or unblinded, extent of loss to follow-up); 2) different doses of calcium supplementation (above and below 800 mg/d, a value that approximates the median dose of calcium supplementation in the eligible trials); 3) type of calcium formulation (a manuscript reviewer suggested this hypothesis); 4) early postmenopausal women (< 5 yr) and late postmenopausal women (5 yr); 5) different levels of baseline calcium intake (less than or greater than 750 mg, a value that approximates the median baseline intake in the eligible trials); and 6) for forearm and hip bone density, subregion of measurement.

6. *Statistical analysis.* For each bone density site (lumbar spine, total body, combined hip, and combined forearm), we calculated the weighted mean difference in bone density between treatment and control groups using the percentage

TABLE 1. Study characteristics from the calcium trials

Study first author year/Ref (primary/secondary prevention)	No. of participants (treatment/control)	Study sample Mean age (SD) BMD g/cm² T score	Baseline dietary calcium intake (SD)	Intervention (Vitamin D supplementation)	Duration (years)	Outcomes measured	Lost to follow-up (%)
Riggs 1998 (23) secondary	119/117	66.3 (2.6) 0.91 g/cm² (0.10) −1.2 0% vertebral fracture prevalence	714 (286) mg/d	Calcium citrate salt 1600 mg vs. placebo	4	BMD: Lumbar spine, total body, total hip; Fractures: vertebral and nonvertebral	59/236 (25%)
Recker 1996 (20) (secondary)	95/104 Fractures 52/42 No fractures 43/62	73.5 (7.1) 0.727g (0.14) 47.7% vertebral fracture prevalence	431 (194) mg/d	Calcium carbonate 1200 mg vs. placebo	4	BMC: Distal forearm; Fractures: vertebral fractures	17/197 (8.6%)
Prince 1995 (19) secondary	42/42	62.5 (4.5) 0.87 g/cm² (0.13) −1.6 Independent of fracture prevalence	804 (299) mg/d	Calcium lactate gluconate 1000 mg vs. placebo also calcium and exercise and milk powder group (not included)	[illegible]	BMD: Total spine, femoral neck, total hip, intertrochanteric, trochanter, ultradistal ankle	13/84 (15.5%)
Aloia 1994 (7) (primary)	38/40	51.8 (1.7) 1.01 g/cm² (0.06) 0.0 0% vertebral fracture prevalence	481 (111) mg/d	Calcium carbonate 600 mg vs. placebo (400 IU vitamin D/d)	3	BMD: Lumbar spine, femoral neck, trochanter, total body, 1/3 radius, Ward's triangle	8/78 (10.3%)
Chevalley 1994 (6) secondary	31/31	72.1 (0.6) 0.98 g/cm² (0.02) 0.6 0% recent hip fracture prevalence	619 (318) mg/d	Calcium carbonate 800 mg vs. placebo or Ossein mineral complex (300,000 IU vitamin D at study start)	1.5	BMD: Femoral neck, femoral shaft; Fractures: vertebral and nonvertebral	10/62 (16.1%)
Strause 1994 (14) secondary	29/28	65.4 (5.3) 0.82 g/cm² (0.13) −1.2 Independent of fracture prevalence	572 (298) mg/d	Calcium citrate malate 1000 mg placebo or trace minerals without calcium	2	BMD: Lumbar spine	3/57 (5.4%)
Reid 1993 (16) primary	68/67	58.0 (5.0) 0.87 g/cm² (0.11) −1.6 0% symptomatic vertebral fracture prevalence	745 (298) mg/d	Calcium 1000 mg vs. placebo	2	BMD: Lumbar spine, proximal femur, total body; Fractures: symptomatic vertebral fractures	[illegible]
Elders 1991 (26) (primary and secondary)	198/97	46–55 0.88 g/cm² (0.13) −1.5 Independent of fracture prevalence	1150 (1082) mg/d	Calcium carbonate 1000 mg or 2000 mg vs. placebo	3	BMD: Lumbar spine	47/295 (15.9%)
Nelson 1991 (21) (secondary)	19/22	60.2 (6.5) 0.96 g/cm² (0.06) −1.1 Independent of fracture prevalence	879 (534) mg/d	Calcium 831 mg and exercise, calcium 831 mg alone, exercise alone or placebo	1	BMD: Lumbar spine, proximal femur and distal radius	5/41 (12.2%)
Prince 1991 (22) (secondary)	39/41	56.0 (4.0) 2.72 mg/mm (31) Independent of fracture prevalence	781 (300) mg/d	Calcium gluconate 1000 mg plus exercise vs. exercise alone	2	BMD: Distal, median and proximal forearm	10/80 (12.5%)
Dawson-Hughes 1990 (15) (primary and secondary)	238/123	58.4 (4.8) 0.91 g/cm² (0.02) −1.3 0% non traumatic fracture prevalence	406 (84) mg/d	Calcium carbonate 500 mg; Calcium citrate malate 500 mg vs. placebo	2	BMD: Lumbar spine, femoral neck, 1/3 radius	46/361 (12.7%)
Smith 1989 (17) (primary)	44/38	55 (4.7) 0.68 g/cm Independent of fracture prevalence	679 (237) mg/d	Calcium 500 mg vs. placebo	4	BMC and BMD: Radius, ulna and humerus	15/82 (18.3%)

TABLE 1. Continued

Study: first author year/Ref primary/secondary prevention[a]	No. of participants treatment/control	Study sample: Mean age (SD) BMD g/cm[2] T score	Baseline dietary calcium intake (SD)	Intervention: Vitamin D supplementation	Duration: years	Outcomes measured	Lost to follow-up %
Hansson 1987 [25] secondary	25/23	66.0 (6.0) 273 mg/mm 100% vertebral fracture prevalence	Not available	Calcium gluconate 1000 mg daily vs. placebo	4	BMC Lumbar spine Fractures vertebral	9/50 (18%)
Reid 1988 [26] primary	15/13	50 (2.8) 0.72 g/cm[2] (0.15) vs. 0 Independent of fracture prevalence	Not collected (800 mg national average)	Calcium carbonate 2000 mg vs. placebo	2	BMD Lumbar Spine total body distal forearm proximal forearm	3/28 (10.7)
Lumker 1989 [27] secondary	20/20	80.0 (3.0) 0.6 mg/mm (2) 100% forearm fracture prevalence	Not collected	Calcium 1000 mg vs. placebo	3	BMC Femoral neck and femoral shaft	1/40 (3)

BMC, Bone mineral content.
[a] Refer to *a priori* hypotheses regarding heterogeneity defining primary and secondary prevention.
[b] BMD g/cm[2] lumbar spine, corrected to Hologic measurements with SD *in parenthesis*.
[c] Perimenopausal women randomized; only postmenopausal women included in analysis; forearm BMC mg/mm; T score not available.

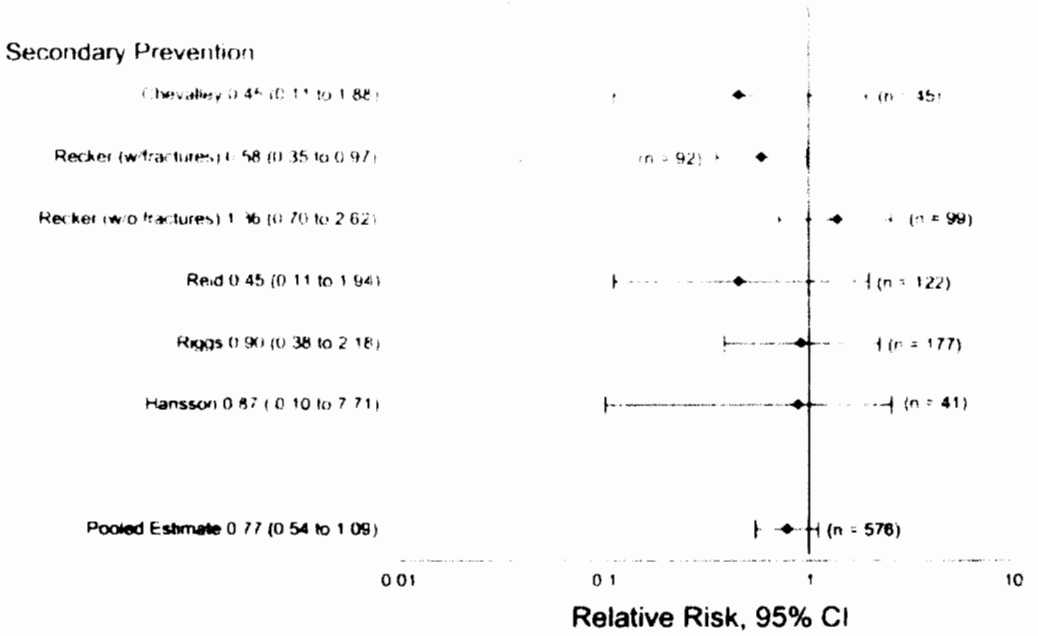


FIG. 2. RR of vertebral fracture after treatment with calcium.

TABLE 2. Weighted RR of fracture After treatment with calcium

Fracture site	No. of trials	Sample size	RR (95% CI)	RR *P* value	Heterogeneity *P* value
Vertebral	5	576	0.77 (0.54,1.09)	0.14	0.40
Non vertebral	2	222	0.86 (0.43,1.72)	0.66	0.54

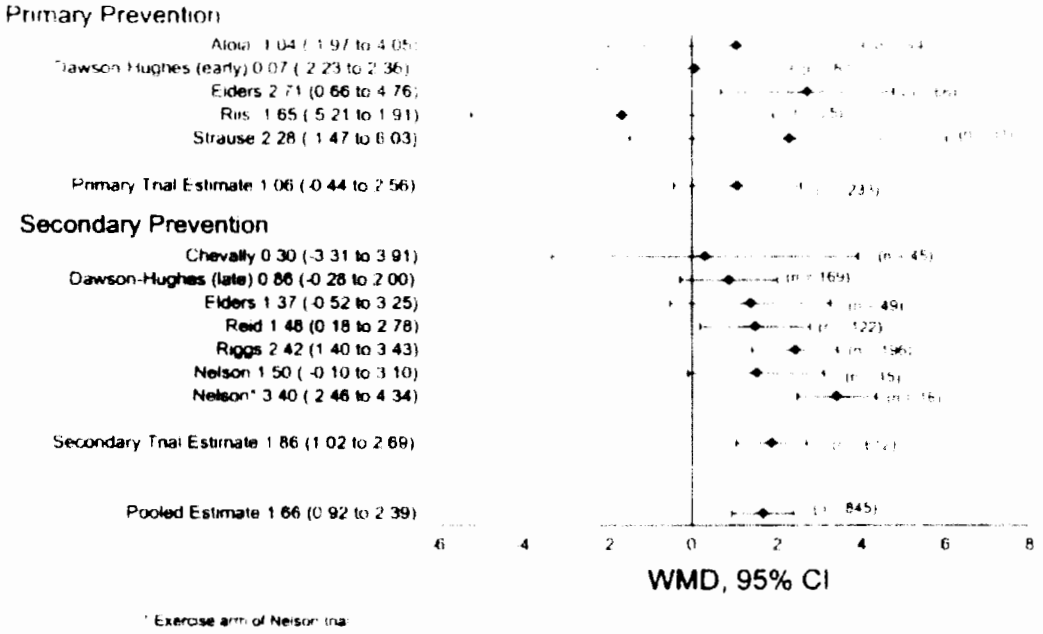
We interpreted $P < 0.05$ as indicating important between study differences in results.

change from baseline in the treatment and placebo groups and the associated SD values. We constructed regression models in which the independent variables were year and dose, and the dependent variable the effect size, and we used this regression to determine the years across which pooling was appropriate. To assess whether the magnitude of heterogeneity (differences in apparent treatment effect across studies) was greater than one might expect by chance, we conducted a test based on the χ^2 distribution with N-1 degrees of freedom, where N is the number of studies (11).

TABLE 4. Weighted mean difference of bone density after treatment with calcium

Bone density site	No. of trials	Sample size (n)	Weighted mean difference (95% CI)	P value	Test of heterogeneity P value
Total body	4	358	2.05 (0.24, 3.86)	0.03	0.01
Lumbar spine (2 yr)	9	845	1.66 (0.92, 2.39)	0.01	0.02
Lumbar spine (3 or 4 yr)	2	218	1.13 (−0.11, 2.38)	0.07	0.71
Combined hip	8	830	1.64 (0.70, 2.57)	0.01	0.04
1/3 Distal radius	6	615	1.91 (0.33, 3.50)	0.02	0.01

We interpreted $P < 0.05$ as indicating important between study differences in results.



* Exercise arm of Nelson trial.

FIG. 3. Weighted mean difference for lumbar spine after treatment with calcium at 2 yr.

For each fracture analysis we calculated a risk ratio (a RR) using methods described by Fleiss (11). We derived risk ratios by constructing two-by-two tables for vertebral and nonvertebral fractures. We tested for heterogeneity using a χ^2 procedure (12).

We tested whether our *a priori* hypotheses could explain variability in the magnitude of treatment effects across studies using a procedure described by Hedges and Olkin (12). To test for publication bias, we constructed plots of the relationship between sample size and the magnitude of the treatment effect.

D. Results

1. Search results. Figure 1 presents the results of our search for eligible studies. Electronic and hand searching uncovered a total of 66 published papers that addressed the relationship between calcium intake and bone mineral density. Twenty-three described RCTs (6, 7, 13–33), of which 7 were excluded for various reasons including combination with vitamin D (29, 33), male participants (31), trial duration less then 1 yr (30, 32), or measurement of bone density exclusively at the ultra-distal forearm site (27, 28).

Of the 16 authors of eligible studies whom we contacted for missing data, 13 provided additional data (6, 7, 13–23). We had to exclude one study due to lack of the data regarding error terms for the analysis (24), and we were unable to contact one investigator (26), although the study provided sufficient data for inclusion. Thus, 15 RCTs both fulfilled our eligibility criteria and provided useful data for pooling (6, 7, 13, 23, 25, 26). Of the 13 investigators who did provide additional data, 11 were able to provide us with all the information we sought (6, 7, 14–20, 22, 23), whereas the other 2 provided us with some of the information we requested (13, 21).

2. Study characteristics. The 15 RCTs included 1806 patients, of whom 953 patients received calcium supplementation. Table 1 summarizes the characteristics of these studies. Of the 15 studies, 13 investigators confirmed that the randomization was concealed (6, 7, 13–23), 13 investigators confirmed that patients, caregivers, and those measuring outcome were blind to allocation (6, 7, 13–23). None of the trials had between 1% and less than 5% loss to follow-up, 13 trials had a loss to follow-up between 5% and 20%, and 2 trials lost

TABLE 4. Heterogeneity of difference of bone mineral density

Bone density site	Heterogeneity *P* value	Primary–secondary difference (95% CI; *P* value)	Less to follow-up (<1 yr – ≥1 yr)	Calcium supplementation (<800 mg – ≥800 mg)	Baseline daily calcium intake (<750 mg – ≥750 mg)	Site measured (Ward's triangle – femoral neck)
Total body	0.01	4.50, 0.59 3.91 (1.18, 6.64) *P* = 0.01	2.91, 0.37 2.54 (−1.06, 6.14) *P* = 0.17	0.63, 5.50 1.87 (−6.80, 2.93) *P* = 0.01	0.82, 2.86 2.05 (−7.12, 3.02) *P* = 0.43	One site only
Lumbar spine 2 yr	0.02	1.06, 1.86 0.80 (−2.51, 0.92) *P* = 0.36	1.32, 2.17 0.35 (−2.24, 0.53) *P* = 0.23	2.00, 0.74 1.27 (0.02, 2.51 *P* = 0.05	1.87, 1.39 0.48 (−0.94, 1.90) *P* = 0.51	One site only
Lumbar spine 3–4 yr	0.71	0.65, 1.25 0.60 (−3.76, 2.57) *P* = 0.71	0.65, 1.25 0.60 (−3.76, 2.57) *P* = 0.71	1.25, 0.65 0.60 (−2.57, 3.76) *P* = 0.71	Only 1 subgroup	One site only
Combined hip	0.04	2.78, 1.51 1.27 (−3.04, 6.57) *P* = 0.64	1.78, 1.35 0.43 (−1.41, 2.10) *P* = 0.73	1.53, 2.11 0.57 (−3.28, 2.14) *P* = 0.68	1.55, 1.70 0.14 (−2.15, 1.86) *P* = 0.89	1.37, 1.87 0.50 (−2.16, 1.16) *P* = 0.55
1/3 Distal radius	0.01	2.51, 1.71 0.81 (−1.80, 3.41) *P* = 0.54	1.70, 3.43 1.74 (−4.55, 1.06) *P* = 0.22	2.30, 1.18 1.12 (−1.54, 3.78) *P* = 0.41	1.05, 2.35 1.30 (−4.70, 2.10) *P* = 0.45	One site only

We interpreted $P = 0.05$ as indicating important between-study differences in results.

TABLE 5. Difference of bone mineral density by calcium type

Bone density site	Heterogeneity *P* value	Calcium citrate–calcium carbonate difference (95% CI; *P* value	Calcium citrate–calcium gluconate difference (95% CI; *P* value	Calcium carbonate–calcium gluconate difference (95% CI; *P* value
Total body	0.01	0.37, 4.36 4.13 (1.33, 6.93) *P* = 0.01		
Lumbar spine 2 yr	0.44	2.41, 1.24 1.17 (−0.43, 2.77) *P* = 0.15		
Lumbar spine 3 yr or 4 yr	0.71	1.25, 0.65 0.60 (−2.57, 3.76) *P* = 0.71		
Combined hip	0.15	1.15, 4.19 3.03 (0.92, 5.13) *P* = 0.03	1.15, 1.61 0.46 (−2.17, 1.26) *P* = 0.60	4.19, 1.61 2.58 (−0.31, 5.48) *P* = 0.08
1/3 Distal radius	0.46	2.83 Only 1 subgroup		

more that 20% of their patients. We were unable to obtain the methodology information for two of the trials (25, 26).

3. Fractures. Five studies including 576 women reported fractures as an outcome (6, 16, 20, 23, 25). All five trials investigated the influence of calcium supplementation on vertebral fractures. The pooled RR indicated a nonsignificant trend toward reduction in vertebral fractures in the calcium group (RR 0.77, 95% CI 0.54–1.09, $P = 0.14$; Fig. 2). The two trials (6, 23) that reported nonvertebral fractures had very few events, and the CI on the pooled estimate is therefore very wide (RR 0.86, 95% CI 0.43–1.72, $P = 0.66$). For both vertebral and nonvertebral fractures, the effect of calcium was consistent across trials (heterogeneity $P = 0.40$, 0.54, respectively; Table 2). The funnel plots provided no evidence of publication bias.

4. Bone mineral density. Table 3 summarizes the impact of calcium on bone mineral density at the four sites we examined. Our initial analyses suggested that we could pool across years in all instances but one, the lumbar spine. Here, the estimated effect of calcium for yr 3 and 4 was actually less than for yr 1 and 2 (Table 3). For all sites but lumbar spine at 2 yr of follow-up (Fig. 3), calcium showed an effect of between 1% and just over 2% in bone density.

At all sites, we found considerable variability in estimates of effect across trials reflected in statistically significant tests of heterogeneity. Funnel plots provided no persuasive evidence of publication bias.

Our search for explanations of this heterogeneity proved largely fruitless (Table 4). For the total body measurement we observed a statistically significantly greater effect in primary than secondary studies, and with smaller doses of calcium than larger doses. For lumbar spine at 2 yr, the effect was in the opposite direction, suggesting a larger impact of higher doses.

We did find an apparently greater effect of calcium carbonate than calcium citrate on total body bone density and on the hip site (Table 5). However, the trend for the lumbar spine measurements was in the opposite direction (larger effects with calcium citrate). Moreover, the total body and hip site analyses were based on only a single RCT using calcium citrate and two RCTs using calcium carbonate. Thus, any inferences based on this analysis are extremely weak. No other subgroup analysis showed statistically significant results.

F. Discussion

This systematic review is restricted to calcium supplementation with minimal vitamin D. Large studies of vitamin D have shown conflicting results (29, 33). We summarize the data from all randomized trials of vitamin D in *Section VIII*.

Our data suggest a relatively small, but possibly important, effect of calcium supplementation on bone density in postmenopausal women. The inference that calcium increases bone density is strengthened by the consistency of the finding across four sites of measurement (Table 3). The inference is, however, weakened by the large loss to follow up in most studies (Table 1) and by the unexplained heterogeneity of results across studies (Tables 3 and 4).

To establish the effect of calcium supplementation on fractures would require large, relatively long trials measuring fracture incidence. We found only five RCTs that measured fracture rate. The point estimate from the meta-analysis of these five studies suggested a potentially important reduction in vertebral fractures (RR 0.77, 95% CI 0.51–1.09, $P = 0.14$, RR 0.77), and a smaller reduction in risk of nonvertebral fractures (RR 0.86, 95% CI 0.43–1.72, $P = 0.66$). Thus, even for vertebral fractures, a true underlying substantial reduction in the RR of fractures (46%) or small increase in the RR of fractures (10%) both remain plausible.

The estimates provided by our analysis are limited by problems inherent in the original studies, including a lack of uniformity in outcome measures. In 1996, during the Conference on Outcome Measures in Rheumatology Clinical Trials (OMERACT 3), participants agreed on a potential core set of outcome measures for osteoporosis (34). A core set will permit the comparison of data across all trials to perform accurate meta-analyses. The primary outcomes will be the number of women experiencing new nonvertebral and vertebral fractures (clinical and radiographic), bone mineral density, and toxicity (measured by withdrawals and side effects), as recommended by the OMERACT group in 1997 (34).

As well as considering these issues, future investigations should take care with the selection of study patients, the dose and formulation of calcium administration, and the measures of outcome. When they select study populations, investigators should also consider factors that may influence the effectiveness of calcium supplementation, including age, years since menopause, dietary calcium intake, and vitamin D status, in selecting study populations. Site of bone density measurement, type and precision of the instruments, and definition of fracture may also influence the apparent magnitude of treatment effects.

In summary, we found small but statistically significant and potentially important effects of calcium supplementation on bone loss over a 2-yr period. Ensuring adequate calcium intake may be important for a variety of reasons, including its role as part of an intervention that includes another agent such as vitamin D or bisphosphonates. The magnitude of reduction in fracture risk with calcium supplementation alone remains an open question.

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Beneficial Effect of Calcium Derived from *Lithothamnion corallioides* on Markers of Calcium Metabolism in Pre-Menopausal Women

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ABSTRACT

Objective: This pilot study tested the hypothesis that calcium derived from a novel botanical source could demonstrate greater influence over the markers of calcium metabolism when compared to a conventional calcium carbonate supplement.

Design: This study was a double blind crossover trial.

Subjects/Setting/Intervention/Main Outcome Measures: Twelve fasting female subjects received a single oral dose of *Lithothamnion corallioides* (Aquamin F), calcium carbonate or placebo. Blood and urine samples were collected at baseline and over the 12-hour treatment period. Ionized and total calcium and parathyroid hormone (PTH) were measured.

Statistical Analyses Performed: Baseline characteristics and primary outcome variables were compared using paired t-tests and repeated measures ANOVA with Greenhouse-Geisser correction. Statistical significance was established at $p < 0.05$.

Results: Subjects treated with Aquamin F demonstrated significantly greater urinary clearance of calcium after 12 hours as compared to placebo ($p = 0.004$). Following the meal at 90 minutes, subjects treated with Aquamin F demonstrated a more prolonged suppression of serum PTH concentration, remaining significantly lower than placebo at 90, 120 and 240 minutes. Calcium carbonate provided an intermediate response, urinary clearance was not significantly different from placebo treatment and PTH was only significantly lower than placebo at 90 minutes.

Conclusions/Applications: Aquamin F may demonstrate greater influence over bone metabolism than calcium carbonate, as suggested by a greater calciuric response and a more

prolonged suppression of serum PTH concentrations following a meal in pre-menopausal women. Although additional studies are needed, Aquamin F may represent a significant improvement over currently available dietary calcium supplements.

Beneficial Effect of Calcium Derived from *Lithothamnion corallioides* on Markers of Calcium Metabolism in Pre-Menopausal Women

INTRODUCTION

Osteoporosis is a major clinical problem affecting about 44 million Americans with significant physical, psychological, and financial consequences (1-3). The aging of the American population has led to a renewed interest in the risk factors, diagnosis, prevention and treatment of this disease. The average dietary calcium intake is well below the recommended dietary intake (4) and calcium supplementation is widely advocated to achieve the recommended calcium amounts for adolescent and pre-menopausal females (5-7), physically active people (8), postmenopausal and elderly women, (9,10) and for the management of osteoporosis (11,12).

For calcium supplements to be clinically useful the supplements must contain a form of calcium that is readily bioavailable when consumed; however, the bioavailability of different calcium supplements varies widely (13). Bioavailability, the percentage of calcium absorbed from a calcium preparation by human subjects, depends not only on the intrinsic calcium absorptive capacity of the subjects tested (14), but also on calcium absorbability, or the extent to which calcium in a given preparation is available for absorption (10,15,16). Although natural plant sources of calcium are rare the skeletal remains of a species of seaweed, *Lithothamnion corallioides,* is known to contain large amounts of a highly porous and readily absorbable form of calcium. Once harvested, the crude residual product from this seaweed species consists primarily of mineral substances, particularly calcium carbonate (approximately 32% calcium by weight; Table 1). The calcium carbonate found in *Lithothamnion sp.* is currently being evaluated

as a calcium supplement (Aquamin F™ Marigot Group, Ltd, Cork, Ireland). Animal studies demonstrated that Aquamin F was 16% more bioavailable than tri-calcium phosphate and 7% more bioavailable than another form of calcium carbonate (unpublished data; Marigot Group, Ltd).

Measuring the systemic absorption of an oral calcium supplement is made difficult by the fact that calcium is a normal and dynamic constituent of the extracellular milieu. Increased calcium absorption was expected to result in a measurable increase in urinary calcium secretion; however, additional markers were needed to measure increased calcium absorption. One such marker used in this trial was serum parathyroid hormone (PTH) concentration. Several studies have shown that PTH varied in relation to calcium level. Elevated PTH concentrations have been found in postmenopausal women; however, treatment with calcium reduced the PTH concentration to levels seen in premenopausal women (12, 17-20). In addition, consumption of a meal impacted serum PTH levels but the magnitude and direction of this effect varied (21-24).

This study was designed to evaluate the impact of calcium carbonate derived from a novel, botanical source demonstrated on markers of calcium metabolism compared to an existing calcium carbonate supplement or placebo in pre-menopausal women.

METHODS

Study Treatments

The protocol was approved by a commercial IRB and consisted of three treatments (placebo, calcium carbonate, or Aquamin F) separated by a 7-day washout period before the next

74 treatment. Each subject was randomized to one of 6 possible treatment orders in double-blinded
75 fashion. To avoid any possible seasonal effects on calcium metabolism, all subjects completed
76 this study within 4 weeks. For one week prior to each treatment period, subjects were
77 maintained on a diet restricted with respect to calcium (400 mg/day) and sodium (100 mEq /day).
78 On the day prior to the test period, each subject fasted for 12 hours and drank 600 ml of distilled
79 water at 20:00 and 300 ml at 23:00. On the morning of the test day, subjects drank 600 ml of
80 distilled water at 06:00 and then 300 ml every 2 hours during the remainder of the 12-hour test
81 period. Each subject received a standard calcium- and sodium-restricted meal at 90, 360 and 720
82 minutes after administration of the treatment dose. Each treatment dose included three 2-piece
83 gelatin capsules delivering 720 mg of elemental calcium (240 mg/capsule) or placebo (provided
84 by Marigot Group, Ltd., Cork, Ireland).
85
86 **Measurements**
87 Blood samples were collected at 0 (before), and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 hours
88 after administration of the treatment and analyzed for ionized calcium, total calcium, and
89 parathyroid hormone. Urine was collected immediately prior to time 0 and during the entire12-
90 hour test period. Total urine volumes produced during the test period were recorded and urinary
91 calcium was measured. Measurements were performed in a single laboratory (Quest Diagnostics
92 Laboratory, Minneapolis, MN). Total urinary calcium excretion was calculated by multiplying
93 the urinary calcium concentration (mg/dl) by the volume of urine collected. Urinary calcium
94 excretion ratios were calculated for Aquamin F over placebo, calcium carbonate over placebo,
95 and Aquamin F over calcium carbonate.
96

Statistical Analysis

Baseline characteristics and primary outcome variables were compared using paired t-tests and repeated measures ANOVA with Greenhouse-Geisser correction to analyze the three treatment conditions on any outcome variable. Statistical significance was established at $p < 0.05$. Classic pharmacokinetic techniques(24) were used to assess bioavailability. The pharmacokinetic parameters, time to maximum concentration (Tmax), elimination half-life (T½), maximal concentration (Cmax), and area over the curve (AOC) were calculated using the linear trapezoidal rule.

RESULTS

Study Population and Baseline Measurements

Twelve healthy female volunteers gave their written informed consent prior to participation in any trial activities and were enrolled in the study. The subjects were not using any mineral supplement or medication known to affect the metabolism of calcium. All 12 randomized subjects completed the trial with no missing data. The mean age was 28.8 years, mean body weight was 66.7 kg, and mean BMI was 25.5 kg/m.^{2} No serious adverse events were reported in this trial and vital signs were within normal limits throughout the trial. No significant differences were found between the groups for the baseline laboratory and vital sign measurements.

Serum Ionized and Total Calcium

Serum ionized and total calcium concentrations were relatively unchanged over the duration of the 12-hour study period. The ionized calcium concentrations were approximately 5 mg/dL and

the total calcium concentrations were about 9 mg/dL during each of the three treatment periods. Consequently, estimates of Tmax and T½ were not possible because of the flat pharmacokinetic profiles and no estimates of AUC to infinity could be calculated. No significant differences were found between any of the treatment groups for ionized or total serum calcium concentrations.

Urinary Calcium Excretion

In contrast to the relatively unchanged serum calcium values, treatment with Aquamin F resulted in a significantly greater urinary calcium concentration and total amount of calcium excreted than was observed when subjects received placebo ($p = 0.004$ and $p = 0.006$, respectively; Table 2). In contrast, the urinary calcium concentration and total calcium excretion amounts during the calcium carbonate treatment were not significantly different than was observed when subjects received placebo ($p = 0.36$ and $p = 0.95$, respectively; Table 2).

Serum PTH

Table 3 shows the effect of treatment on serum PTH concentration and Figure 1 shows the PTH level relative to baseline (time zero) in order to observe the relative changes in concentration. Relative to time zero, the serum PTH concentrations for all treatments decreased during the first 60 minutes after dosing. At 90 minutes, immediately before consumption of the meal, the serum PTH concentration for the placebo treatment increased back to baseline levels and then decreased with further sampling before returning to baseline levels again at 360 minutes. In contrast to the changes in serum PTH seen with placebo, the serum PTH concentration after the Aquamin F treatment continued to decrease after 60 minutes and remained significantly lower than the PTH concentration for placebo at 90, 120 and 240 minutes ($p = 0.003$, 0.017 and 0.030,

143 respectively). The serum PTH concentration after the calcium carbonate treatment was
144 intermediate between the Aquamin F and placebo responses being significantly decreased only at
145 90 minutes compared to placebo (p = 0.026). All treatments resulted in similar PTH responses
146 after 300 minutes and returned to baseline levels at 360 minutes after dosing, immediately prior
147 to the next meal.
148
149 **DISCUSSION**
150 The number of osteoporosis-related physician visits continues to increase (25) and under-
151 treatment has been noted particularly among elderly patients residing in institutional settings (26,
152 27). Increased calcium intake may be associated with a substantial reduction in the risk of bone
153 fracture (28) and osteoporosis prevention begins with the development of optimal levels of peak
154 bone mass as early as 6-10 years of age and certainly during the second decade of life. Regular
155 exercise and a healthy diet with enough calcium helps young adults maintain good bone health
156 and may reduce their risk of osteoporosis later in life. This pilot study enrolled young, pre-
157 menopausal women in order to study a period of relative bone stability compared to that
158 anticipated for adolescent or post-menopausal women. We measured the pharmacokinetic and
159 pharmacodynamic responses to a novel form of calcium derived from a natural source of
160 mineralized seaweed (*Lithothamnion sp.*; Aquamin F) compared to calcium carbonate or
161 placebo.
162
163 The lack of response in the serum ionized calcium and total calcium after administration of 720
164 mg of elemental calcium is in contrast to previous studies performed in post-menopausal women.
165 The inability to capture this dynamic change in serum calcium levels after oral administration of

166 calcium may be related to the younger age of the subjects, the reduced dose of calcium
167 administered (some studies used an oral dose of 1 gram calcium), the small number of subjects
168 tested, the efficiency of calcium homeostasis in these particular subjects, or the small but real
169 natural variability in serum calcium measured over time in this trial despite a calcium-restricted
170 diet prior to each test period. The relationship between the quantity of calcium absorbed and the
171 pharmacokinetic analysis of calcium is complex and the impact of dietary components on bone
172 biochemistry and osteoporosis is multi-factorial (29-30). Calcium absorption is known to evoke
173 physiologic responses that both reduce the amount of calcium getting into the blood from bone
174 and suppress further absorption of calcium from the intestine (31).
175
176 The amount of calcium excreted in the urine during active calcium treatments (Aquamin F and
177 calcium carbonate) was 50-60% higher than during placebo treatment. The calcium excreted in
178 the urine during the Aquamin F treatment was 6% higher than the calciuric response during the
179 calcium carbonate treatment. The cross over nature of the trial design allowed determination of
180 each individual's response to each treatment and revealed a significant increase in calcium
181 excretion from the Aquamin F treatment compared with placebo (p = 0.004 and p = 0.006,
182 respectively; Table 3). This was not observed with the calcium carbonate treatment.
183
184 Previous research demonstrated a decline in serum PTH levels in response to an increase in
185 serum calcium from oral calcium supplements. (12) In this study, Aquamin F treatment had
186 significantly decreased PTH concentrations compared to placebo at 90, 120 and 240 minutes (p =
187 0.003, p = 0.017 and p = 0.030, respectively) while calcium carbonate treatment had a
188 significantly decreased PTH concentration compared to placebo only at 90 minutes (p = 0.026).

189 PTH concentrations are highly sensitive to the consumption of a meal (21-23). A prior study has
190 shown that ingestion of a gastric acid-stimulating test meal resulted in increased serum PTH in
191 normal subjects and ingestion of antacid with the test meal prevented an increase in serum PTH
192 concentration (21). The greater effectiveness in suppressing PTH concentration shown by
193 Aquamin F compared to calcium carbonate may be a feature of its antacid qualities or its calcium
194 load. Another study showed that an increase in calcium excretion followed consumption of a
195 high protein meal (22) without changes in serum PTH concentration. This may be a consequence
196 of the experimental design as the time of sampling started with consumption of the meal.
197 Changes in PTH may occur in anticipation of a meal as well as being a consequence of its
198 consumption (32)
199
200 This study examined the absorption, PTH response, and renal excretion of calcium in pre-
201 menopausal women treated with Aquamin F, calcium carbonate, and placebo. Calcium from
202 *Lithothamnion sp.* has a highly porous structure, resulting in substantially greater surface area
203 per particle compared to calcium carbonate from other sources (33). The results of this pilot
204 study suggest that Aquamin F is more biofunctional when impacting bone metabolism than a
205 traditional calcium supplement, even though both are calcium carbonate. Oral administration of
206 Aquamin F in premenopausal women had a greater calciuric response and a more profound
207 pharmacodynamic response resulting in a prolonged suppression of serum PTH concentrations
208 following a meal when compared to similar treatments with calcium carbonate or placebo.
209
210 Subsequent studies are needed to evaluate the implications of this research, the impact of
211 Vitamin D on the absorption of Aquamin F and to evaluate the effects of Aquamin F in older,

212 postmenopausal women. Although additional studies are needed, this study suggests that
213 Aquamin F, a novel calcium supplement derived from the seaweed *Lithothamnion sp.*, may
214 represent a new means of providing calcium supplementation to individuals at risk of bone loss
215 due to osteoporosis.
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Table 1

Calcium	32%
Magnesium	3%
Sulphur	0.2%
Potassium	0.10%
Phosphorus	0.08%
Iron	0.05%
Sodium	300 ppm
Manganese	125 ppm
Boron	75 ppm
Zinc	37 ppm
Cobalt	6 ppm
Copper	2 ppm
Selenium	1 ppm

Table 1. Mineral Content of Aquamin F

Table 2

Calcium Concentration in Urine (mg/dL; $N = 12$)															
TREATMENT	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**Mean**	**SD**	**P-value vs. placebo**
Aquamin F	5.8	3.3	7.2	2.9	8.0	10.5	6.2	3.6	5.3	11.6	3.5	3.1	5.92	2.94	0.004
Calcium Carbonate	7.4	2.5	6.6	3.3	12.3	12.0	5.8	8.2	5.7	8.3	3.7	2.4	6.52	3.34	0.36
Placebo	3.1	1.6	3.8	3.0	5.0	7.6	2.7	4.6	4.4	5.0	2.9	2.2	3.83	1.62	---
Calcium Excretion in Urine (mg; $N = 12$)															
TREATMENT	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**Mean**	**SD**	**P-value vs. placebo**
Aquamin F	105.9	65.0	156.6	55.1	144.0	196.9	69.8	129.6	135.2	185.6	56.0	68.2	114.0	51.1	0.006
Calcium Carbonate	115.4	52.5	122.1	85.0	199.9	150.0	78.3	196.8	153.9	91.3	85.1	47.4	114.8	51.2	0.95
Placebo	49.6	46.8	53.2	64.5	105.0	150.1	41.9	126.5	97.9	78.8	59.5	50.6	77.0	35.2	---

Table 2. Calcium Concentration and Excretion in Urine

Formatted

Table 3

Serum Parathyroid Hormone (mg/dL; $N = 12$)															
Matched pair analysis at 90 minutes															
TREATMENT	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**Mean**	**SD**	**P-value vs. placebo**
Aquamin F	20	21	23	29	37	27	39	30	44	32	28	25	29.6	7.4	0.003
Calcium Carbonate	21	47	20	28	30	27	37	34	31	44	30	35	32	8.1	0.026
Placebo	34	43	39	25	75	33	60	43	37	45	33	39	42.2	13.4	---
Matched pair analysis at 120 minutes															
Aquamin F	26	18	29	38	37	33	43	22	35	22	24	32	29.9	7.7	0.017
Calcium Carbonate	21	29	39	42	30	31	55	28	28	36	27	28	32.8	9.0	0.098
Placebo	38	34	37	35	60	25	72	39	41	31	23	32	38.9	14.0	---
Matched pair analysis at 240 minutes															
Aquamin F	30	25	19	28	34	29	33	20	33	23	28	28	27.5	4.9	0.030
Calcium Carbonate	27	24	27	31	22	28	32	24	28	27	30	34	27.8	3.5	0.056
Placebo	32	36	31	25	42	28	47	31	25	31	29	34	32.6	6.5	---

Table 3. Serum parathyroid hormone analysis at 90, 120 & 240 minutes after dose

Figure 1

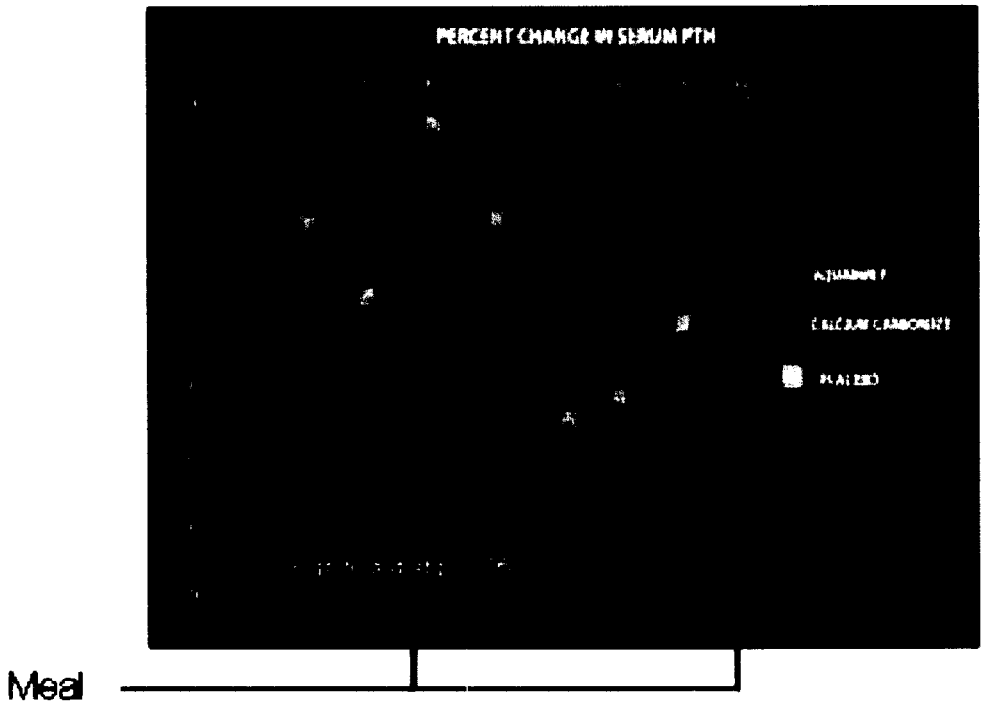

Figure 1: Percent change in serum PTH levels over time.

Compared to placebo, the decrease in PTH concentration following Aquamin F treatment was significant at 90, 120 and 240 minutes ($p = 0.003$, $p = 0.017$ and $p = 0.030$, respectively) while the calcium carbonate treatment was significantly different from placebo treatment only at 90 minutes ($p = 0.026$)

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Chemical and physical characterization of calcified red algal deposits known as maërl

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Abstract

Maërl, comprised of shallow, subtidal deposits of calcareous red algae belonging to the family Corallinaceae, is used in agriculture primarily to increase soil pH. Its use has been strongly criticised because of its high price compared to limestone. The chemical and physical characteristics of maërl and limestone are compared to determine whether these indicate if any benefit is to be gained with the use of the former. Analysis by inductively coupled plasma emission spectrophotometry shows that the proportion of magnesium in maërl is about ten times higher than that in the limestone samples tested. The levels of iron, boron and especially strontium are noticeably higher in the calcified seaweed than in the limestone, although the manganese contents are lower. Scanning electron microscopy shows that the surface characteristics of maërl and limestone are similar but, in section, maërl is considerably more porous because of its cellular structure. Atomic force microscopy revealed minor differences in fine structure between the two. The differences between maërl and limestone would not appear to compensate for the considerably higher costs involved with the utilization of the former material.

Introduction

Dead deposits of several detached, calcareous red algae of the family Corallinaceae, known collectively as maërl, are used on acid soil to increase its pH (Blunden et al., 1975). Maërl has been collected from the Cornish coast of England for use in agriculture from at least the 18th century but it would appear that it was not utilised in this way in France until the beginning of the 19th century. Maërl has been obtained commercially for many years by dredging from around the coasts of Brittany in France and off Falmouth Harbour in England (Blunden et al., 1975). Recently the material has also been harvested from Bantry Bay, Co. Cork, Ireland. The species most commonly found in the commercially-harvested maërl beds are *Phymatolithon calcareum* (Pallas) Adey & McKibbin and *Lithothamnion corallioides* P. Crouan & H. Crouan.

Analysis of maërl has shown that it is composed primarily of calcium and magnesium carbonates, with the calcium content, calculated as Ca^{2+}, ranging from 25 to 28% of the dry weight and the magnesium content, calculated as Mg^{2+}, ranging from 1.7 to 3.3%. The major component of the algal material is calcite with magnesium carbonate present as a solid solution in the calcite structure at a concentration of about 8%. Algal tissue comprised about 10–15% w/w of the total material (Blunden et al., 1977).

Agricultural advisors, for example, Gately & Murphy (1994) have been highly critical of the use of maërl because of its cost, but there are farmers who insist that the calcified seaweed has distinct advantages over

Figure 2. Scanning electron microscope picture of a broken maërl piece

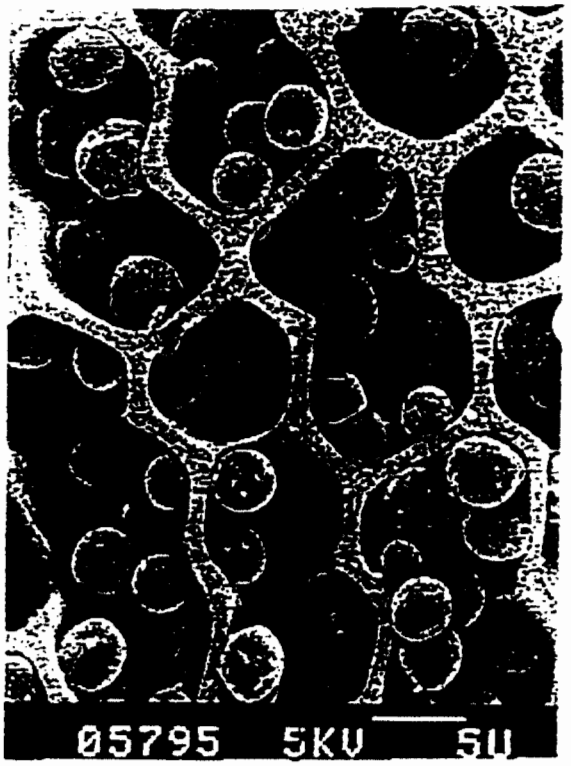

Figure 3. Scanning electron microscope picture of a TS of maërl.

powdered limestone, although these have not yet been established in the scientific literature. If these claims are valid, it would probably be the result of differences in the chemical and physical compositions of the two types of product; these are reported in this communication.

Materials and methods

Maërl samples were obtained from Falmouth Harbour, England by SCUBA diving. Samples from Bantry Bay, Co. Cork, Ireland, were supplied by Celtic Sea Minerals Ltd, Cork, Ireland, who also provided one of the two limestone samples. The second was obtained from Emsworth, Hampshire.

Representative samples of maërl and limestone were dried at 100 °C prior to crushing. The powdered material was then dissolved in Ultra Grade acid (HNO_3/HCl) and analysed by inductively coupled plasma emission spectrophotometry (Fisons Simultaneous Sequential ICP Spectrophotometer – Model 3580).

Atomic force microscopy (AFM) was performed with a Discoverer Topometrix TM 2000 scanning probe microscope (Topometrix Corporation, Saffron Waldron, Essex, UK) using a 70 μm scanner. Imaging was performed in the contact mode under 1-propanol (Aldrich, 99+% spectroscopic grade) using forces in the range 1–40 nN. Standard profile silicon nitride tips were used for imaging and output was displayed on a monitor with a resolution of 400 lines × 400 pixels. Images were levelled by plane fitting and left shading was used to enhance topographic features.

Specimens of limestone and dried maërl were examined by scanning electron microscopy (SEM). Standard aluminium specimen stubs were coated with a thin film of conducting carbon cement which was allowed to dry partially before the specimens were put in place. This step prevented the liquid cement from being drawn into the porous specimens. Limestone was sprinkled onto the surface of the cement. Pieces of maërl were snapped by hand to reveal a clean inner surface and placed on end in the cement with the fresh surface uppermost to observe the cross section. Once the cement was dry, the specimens were sputter coated

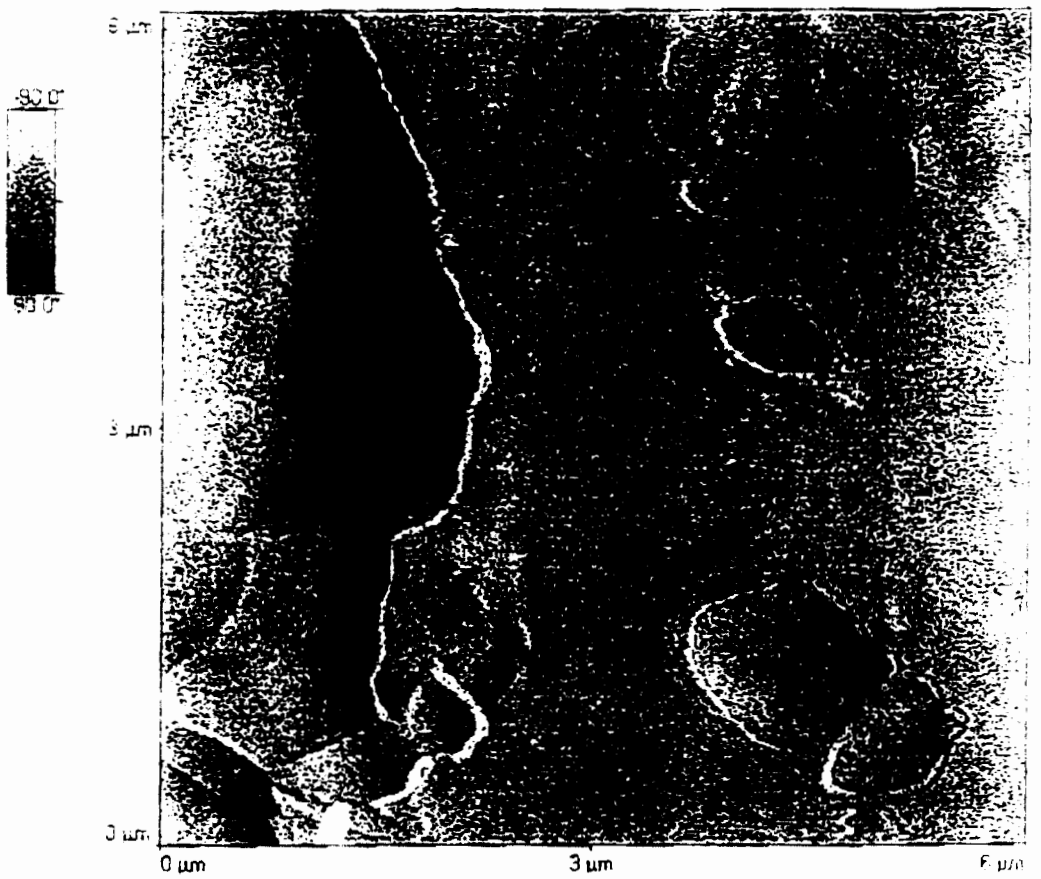

Figure 4 Atomic force microscope picture of limestone surface

with gold and examined in a Hitachi S450 scanning electron microscope using an accelerating voltage of 10 kV

Results

The elemental composition of three maërl and two limestone samples were determined by inductively coupled plasma emission spectrophotometry. The results are presented in Table 1. Although the compositions were generally similar, significant differences were observed. The relative amounts of calcium and magnesium varied, with the milled limestone containing approximately 38% calcium and 0.3% magnesium, whereas the respective values for the maërl samples were 33.3 to 33.8% and 2.33 to 3.33%. The sodium levels of maërl were also higher than those of the limestones

Major differences in the trace element contents of the two types of product were seen for iron, manganese, strontium and boron. The levels of iron, strontium and boron were considerably higher in the calcified seaweed than in the limestone samples, whereas the manganese contents were significantly lower. The largest difference was in the strontium levels of the two products with the content of maërl (1680 to 2190 mg kg^{-1}) being about eight times higher than that of limestone (220 to 242 mg kg^{-1}).

Examination of the outer surface characteristics of pieces of maërl (Figure 1a, b) and limestone (Figure 1c, d) by scanning electron microscopy did not reveal major differences between the two. Both appeared to have a granular, crystalline structure. However, when maërl pieces were broken to reveal their inner structure and this compared with limestone, the porous nature of maërl could be readily observed (Figure 2). Under high magnification (×7000), the

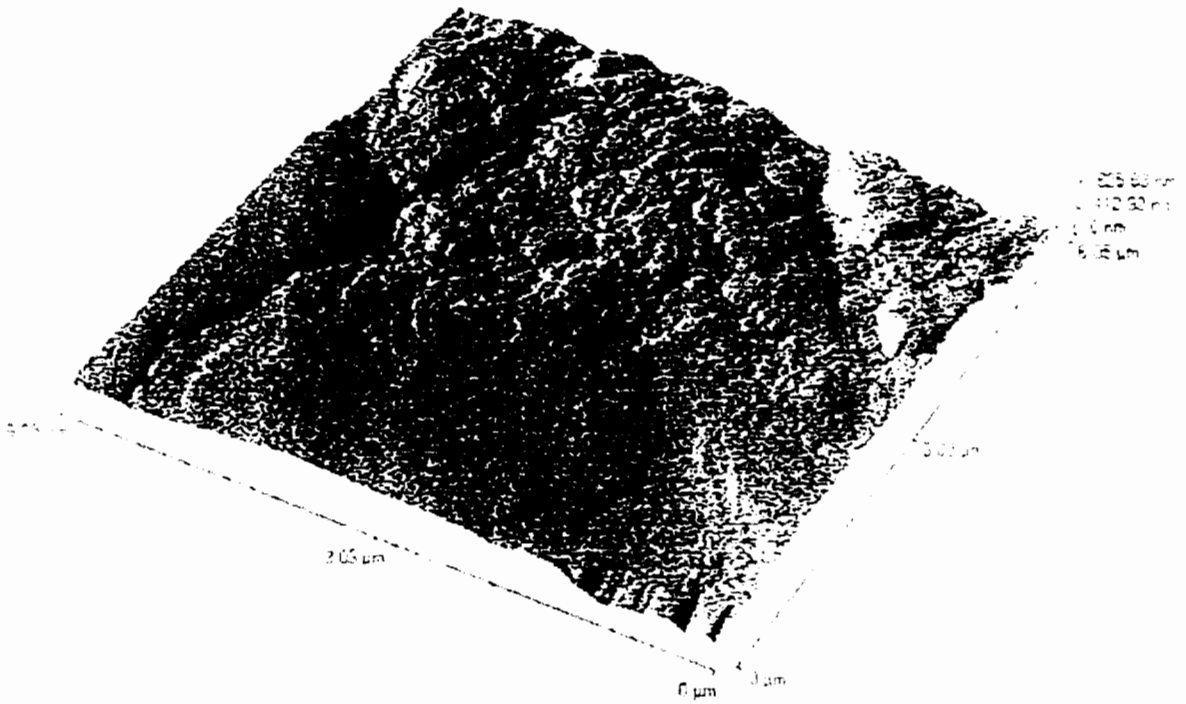

Figure 5. Atomic force microscope picture of maërl surface – three dimensional image

cell structure is noticeable with prominent calcified cell walls with a definite crystalline appearance. In some samples, the cells contained globular inclusions, many of which appeared to be attached to the cell walls by stalks (Figure 3). These particles were quickly stained by both iodine solution and Periodic Acid Schiff reagent.

The maërl and limestone samples were examined by high-resolution imaging using atomic force microscopy (AFM) in the hope that at higher resolution than that available using SEM, significant differences in surface morphology would be detected. In the first experiments, attempts were made to image the ground calcified seaweed and limestone directly. However, this was not possible because of the roughness of the deposits and their mobility during scanning. Hence, sections of maërl and limestone were obtained and imaged either in air or under a layer of 1-propanol.

Figure 4 shows the typical structure of the limestone surface, in which a central smooth and relatively flat area bordered by a higher rougher section can be observed. The corresponding surface roughness profile (not shown) confirmed the flat nature of the central region.

Figure 5 shows the appearance of the surface of maërl imaged under 1-propanol. Both smooth and rougher areas with a fissure type structure are present. The corresponding surface roughness profile of this area (not shown) shows an average peak to peak roughness of approximately 180 nm and the fissure or valley structure has a depth of approximately 190–205 nm.

In general, limestone appeared to possess a much greater macroroughness, although plateau-type regions, not found in maërl, were also present. Maërl could only be imaged under 1-propanol, which may be related to the presence of loosely bound particulate matter. Imaging showed regions of macro and microroughness. Neither maërl nor limestone showed the presence of micropits.

Discussion

The globular inclusions seen in some of the cells of the calcified algae have been observed previously, but their identity was in doubt. Borowitzka et al. (1974) suggested that they were chloroplasts and Alexandersson (1977), that they were endophytic green algae. Garbary

Table 1 Elemental composition of maërl and limestone samples determined by inductively coupled plasma emission spectrophotometry (ICP AES)

Elemental analysis	Maërl samples			Limestone samples	
	Ireland	Ireland	England	Ireland	England
MAJOR ELEMENTS (% wt, dried sample)					
Calcium	33.6	35.3	35.8	38.5	37.8
Magnesium	3.33	2.50	2.33	0.42	0.31
MINOR ELEMENTS (mg kg^{-1} dried sample)					
Sulphur	2800	2800	3500	1100	1600
Phosphorus	500	500	500	740	700
Potassium	350	590	800	290	<100
Sodium	4700	5200	4700	130	<100
Iron	800	2160	1760	710	740
Aluminium	500	760	1500	220	90
Manganese	100	130	127	420	400
Tin	<5	6	31	<5	<20
Indium	5	6	N/A	<5	N/A
Strontium	1190	2080	1680	242	220
Boron	29	29	27	5	<5
Lead	<5	10	16	5	15
Titanium	7	15	2	8	18
Copper	2	3	<2	3	<5
Zinc	16	59	52	170	35
Nickel	<5	<5	<10	<5	<10
Arsenic	<20	<20	<50	<20	<50
Chromium	7	9	9	<5	<5
Cobalt	<5	<5	10	<5	21
Silver	<2	<2	<5	<2	<5
Molybdenum	<20	<20	<20	<20	<20
Loss on drying at 100°C (% wt)	0.25	0.39	0.65	<0.01	0.03

N/A not assayed. Chlorine, Bromine and Iodine not available by ICP-AES

and Veltkamp (1980) noted these globular structures in *Lithophyllum incrustans* Phil., *Lithothamnion glaciale* Kjellm., *L. corallinoides* and *Mesophyllum lichenoides* (L.) Lemoine, and proposed that they were endophytic bacteria. Similar structures were reported for vegetative cells of *Phymatolithon repandum* (Foslie) Wilks & Woelkerling and *P. masonianum* Wilks & Woelkerling by Wilks and Woelkerling (1994), who described them as starch grains. This last proposal appears to be valid as the grains are easily stained with both iodine solution and Periodic Acid Schiff reagent.

Answering the question whether the preferential use of maërl as opposed to limestone (lime) in agriculture is justified, based on the data presented, is difficult. Comparison of the results obtained for maërl and the two limestone samples shows significant differences between the two. The most obvious is in the chemical composition, with maërl having a significantly higher level of magnesium than that of the limestones studied. However, other limestones have calcium and magnesium contents comparable to those of maërl and some, such as dolomite, would have higher levels of magnesium than those of maërl (Boggs 1987). Comparison of the calcium and magnesium contents of maërl reported by us here with those previously recorded (Blunden et al., 1977) show that the relative proportions of the two elements are consistent, whereas for limestone the proportions will vary considerably depending on the source. This relative consistency in maërl may be advantageous, but would not seem to justify a price often ten times that of limestone. It is also unlikely that the differences in trace element contents of the two

types of product would justify the use of the calcified seaweed. The trace element contents of limestones will also vary depending on their source (Boggs, 1987)

The maërl and limestone samples used in this study have different physical characteristics. If small pieces of calcified seaweed were added to the soil, its more porous nature, in comparison to equivalent sized pieces of limestone, might lead to enhanced microbial colonization, more ready breakdown and a speedier effect on soil pH. However, if the two products were applied in a powdered state, this difference is unlikely to be of major significance. Overall, there appears to be insufficient difference in either the chemical or physical characteristics of maërl and limestone to recommend the use of the former in preference to the latter based on the large price differential. However, before a decision can be taken, field trials are required to compare the effects of maerl and limestone on plant growth, crop yield and soil pH to ascertain whether the former has significant advantages over the latter. Unless these are demonstrated, the use of maërl would appear to be unjustified on the basis of its high cost

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