PETITION FOR HEALTH CLAIMS:

2771 '03 MAY 29 P1:16

- GLUCOSAMINE AND OSTEOARTHRITIS
- CHONDROITIN SULFATE AND OSTEOARTHRITIS
- GLUCOSAMINE AND CHONDROITIN SULFATE AND OSTEOARTHRITIS
- GLUCOSAMINE AND OSTEOARTHRITIS-RELATED JOINT PAIN, TENDERNESS AND SWELLING
- CHONDROITIN SULFATE AND OSTEOARTHRITIS-RELATED JOINT PAIN, TENDERNESS AND SWELLING
- GLUCOSAMINE AND CHONDROITIN SULFATE AND OSTEOARTHRITIS-RELATED JOINT PAIN, TENDERNESS AND SWELLING
- GLUCOSAMINE AND JOINT DEGENERATION
- CHONDROITIN SULFATE MAY AND JOINT DEGENERATION
- GLUCOSAMINE AND CHONDROITIN SULFATE AND JOINT DEGENERATION
- GLUCOSAMINE AND CARTILAGE DETERIORATION
- CHONDROITIN SULFATE AND CARTILAGE DETERIORATION
- GLUCOSAMINE AND CHONDROITIN SULFATE AND CARTILAGE DETERIORATION

SUBMITTED TO THE FOOD AND DRUG ADMINISTRATION
MAY 29, 2003

PETITIONER:
WEIDER NUTRITION INTERNATIONAL, INC.

4045B1-08

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BACKGROUND OF THE PETITIONER

A Utah corporation, Weider Nutrition International, Inc. ("Weider") is one of the largest suppliers of health, fitness, and wellness products worldwide. Weider manufactures and markets products in the sports nutrition, bottled drink, diet, natural vitamin, and nutritionally based snack bar categories, including some dietary supplements that contain glucosamine and chondroitin sulfate. Weider has been a health, fitness and sports nutrition leader for sixty years since its founding in 1939.

May 29, 2003

PETITIONER: Weider Nutrition International, Inc.

ADDRESS: c/o Emord & Associates, P.C.
5282 Lyngate Court
Burke, VA 22015

SUBJECT: Petition for Health Claims: Glucosamine and Chondroitin Sulfate and (1) Osteoarthritis; (2) Osteoarthritis-related joint pain, joint tenderness, and joint swelling; (3) joint degeneration; and (4) cartilage deterioration

Food and Drug Administration
Office of Nutritional Products, Labeling, and Dietary Supplements
HFS-800
5100 Paint Branch Parkway
College Park, MD 20740

I. Introduction and Statement of Purpose

Weider Nutrition International, Inc. (hereinafter "Petitioner"), pursuant to Section 403(r)(5)(D) of the Federal Food, Drug and Cosmetic Act ("FDCA")(21 U.S.C. § 343(r)(5)(D)), submits this petition for health claims concerning the relationship between the consumption of glucosamine and chondroitin sulfate and reduction in the risk of: osteoarthritis; osteoarthritis – related joint pain, joint tenderness, and joint swelling; joint degeneration; and cartilage deterioration. The proposed claims are contained in section V below. Attached hereto, and constituting a part of this petition, are all of the items specified in 21 C.F.R. § 101.70(f).

This petition presents a logical and valid evaluation of the scientific studies and clinical trials concerning the relationship between glucosamine and chondroitin sulfate and reduction in the risk of: osteoarthritis; osteoarthritis-related joint pain, joint tenderness, and joint swelling; joint degeneration; and cartilage deterioration. The attached scientific studies demonstrate that the consumption of glucosamine and chondroitin sulfate may reduce the risk of osteoarthritis; may reduce the risk of osteoarthritis-related joint pain, joint tenderness, and joint swelling; and may reduce the risk of joint degeneration and cartilage deterioration. The scientific evidence

justifies permitting a health claim that links consumption of glucosamine and chondroitin sulfate with reduction in those risks. See Glade Report attached as Exhibit (Exh.) 1.

The proposed health claims respond to major public health concerns in the United States: osteoarthritis; osteoarthritis-related joint pain, joint tenderness, and joint swelling; and joint degeneration and cartilage deterioration. 21 C.F.R. § 101.75. Osteoarthritis (also known as degenerative joint disease) is a frequent cause of physical disability among adults, affecting more than 20 million Americans. National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Handout on Health: Osteoarthritis, www.niams.nih.gov/hi/topics/arthritis/oahandout.htm (attached as Exhibit 2). It is the most common form of arthritis. Id. In the US, among those aged 15 to 40 years, the incidence of osteoarthritis in at least one joint is 5%; in those over 65 it is over 60%. Exh. 1 at 7. By 2030, 20 percent of Americans—about 70 million people—will have passed their 65th birthday and will be at risk for osteoarthritis. Exh. 2. The prevalence of at least one mildly symptomatic osteoarthritis joint occurs in about 30% of the U.S. population. Exh. 1 at 7.

Glucosamine and chondroitin sulfate are naturally-occurring substances present in cartilage and the extracellular matrix of the articular cartilage of humans and other mammals. Both substances are commonly sold as dietary supplements. As discussed below, both glucosamine and chondroitin sulfate possess properties that help promote and maintain the structure and function of joints in the body and may also have anti-inflammatory activity. PDR for Nutritional Supplements at 94, 187 (attached as Exh. 3). The scientific studies described in this petition directly address the important public health issue of glucosamine and chondroitin sulfate's effects on osteoarthritis; on osteoarthritis-related joint pain, joint tenderness, and joint

swelling; on joint degeneration; and on cartilage deterioration and further national and DHHS policies by identifying low cost means of reducing risks of those diseases and disease conditions.

The Petitioner believes that the truthful and succinct health information conveyed by its proposed health claims will enable consumers to make prudent and effective dietary choices. Labeling dietary supplements with the proposed glucosamine and chondroitin sulfate claims will inform consumers at the point of sale of current scientific evidence concerning means to reduce the risk of: osteoarthritis; osteoarthritis-related joint pain, joint tenderness, and joint swelling; joint degeneration; and cartilage deterioration. The petitioned claims will accurately impart to consumers scientific understanding about the relationship between glucosamine and chondroitin sulfate and those diseases and disease conditions.

Consistent with the decision in Pearson v. Shalala, 164 F.3d. 650 (D.C.Cir. 1999), reh'g denied en banc, 172 F.2d 72 (D.C.Cir. 1999); Pearson v. Shalala, 130 F.Supp.2d 105 (2001), recon. denied, Pearson v. Thompson, 141 F. Supp. 2d 105 (D.D.C. 2001); and Whitaker v. Thompson, 2002 U.S. Dist. LEXIS 25299 (D.D.C. 2002), the Petitioner respectfully requests that if the agency finds that any of the proposed claims does not satisfy its "significant scientific agreement" standard, that the agency authorize that claim or those claims nevertheless, with such succinct and accurate disclaimers as are reasonably necessary to avoid a potentially misleading connotation. The petitioner will accept any reasonable, succinct, and accurate disclaimers that achieve that objective.

II. Preliminary Requirements

A. Glucosamine and chondroitin sulfate meet the definition of 21 C.F.R. 101.14(a)

The Petitioner seeks FDA approval of the proposed claims for use on dietary supplements containing glucosamine and chondroitin sulfate. Glucosamine and chondroitin sulfate each meet the definition of a "substance" under 21 C.F.R. § 101.14(a): "Substance means a specific food or component of food, regardless of whether the food is in conventional food form or a dietary supplement that includes vitamins, minerals, herbs, or other similar nutritional substances." 21 C.F.R. §101.4 (2002). As stated by NIH, both glucosamine and chondroitin sulfate are "nutrients... found in small quantities in food and are components of normal cartilage." Exh. 2 at 16.

Glucosamine is an amino monosaccharide commonly found in chitin, glycoproteins, and glycosaminoglycans such as hyaluronic acid and heparan sulfate. Exh. 3 at 186. D-Glucosamine is present in all foods containing cartilage or glycoproteins. Exh. 1 at 21. Glucosamine is available commercially as a dietary supplement in three forms: glucosamine hydrochloride or glucosamine HCl, glucosamine sulfate, and N-acetyl-glucosamine. Exh.3 at 187. The type of glucosamine found in supplements is typically derived from marine exoskeletons although synthetic glucosamine is also available. Id. at 187.

Chondroitin sulfate belongs to a family of heteropolysaccharides called glycosaminoglycans. Id. at 93. Glycosaminoglycans in the form of proteoglycans make up the ground substance in connective tissues' extracellular matrix. Id. Chondroitin sulfate is found in human, fish, and shark cartilage; skin; heart valves; tendons; and arterial walls. Id. The sources

of chondroitin sulfate used in supplements include bovine trachea, pork byproducts (ears and snout) and shark cartilage. Id. at 94.[1]

Because glucosamine and chondroitin sulfate are present as foods and components of foods, they are "substances" as defined by 21 C.F.R. § 101.14(a).

B. Glucosamine and chondroitin sulfate meet the definition of 21 C.F.R. 101.14(b)

The proposed health claims meet the relevant eligibility requirements of 21 C.F.R. § 101.14(b). Section 101.14(b) requires:

> (b) Eligibility. For a substance to be eligible for a health claim:
> (1) the substance must be associated with a disease or health-related condition for which the general U.S. population, or an identified U.S. population subgroup (e.g., the elderly) is at risk, or, alternatively, the petition submitted by the proponent of the claim otherwise explains the prevalence of the disease or health-related condition in the U.S. population and the relevance of the claim in the context of the total daily diet and satisfies the other requirements of this section.
> (2) If the substance is to be consumed as a component of a conventional food at decreased dietary levels, the substance must be a nutrient listed in 21 U.S.C. § 343(q)(1)(C) or (q)(1)(D), or one that the Food and Drug Administration (FDA) has required to be included in the label or labeling under 21 U.S.C. § 343(q)(2)(A); or
> (3) If the substance is to be consumed at other than decreased dietary levels:
> (i) The substance must, regardless of whether the food is a conventional food or a dietary supplement, contribute taste, aroma, or nutritive value, or any other technical effect listed in § 170.3(o) of this chapter, to the food and must retain that attribute when consumed at levels that are necessary to justify a claim; and
> (ii) The substance must be a food or a food ingredient or a component of a food ingredient whose use at the levels necessary to justify a claim has been demonstrated by the proponent of the claim, to FDA's satisfaction, to be safe and lawful under the applicable food safety provisions of the Federal Food, Drug and Cosmetic Act.

1. Glucosamine and chondroitin sulfate are associated with a disease for which the general U.S. population is at risk

A "disease or health-related condition" means "damage to an organ, part, structure, or system of the body such that it does not function properly (e.g. cardiovascular disease), or a state

[1] Petitioner's source of chondroitin sulfate is bovine trachea, exclusively obtained from animals bread and harvested in the United States.

of health leading to such dysfunctioning (e.g. hypertension); except that diseases resulting from essential nutrient deficiencies (e.g., scurvy, pellagra) are not included in this definition (claims pertaining to such diseases are thereby not subject to § 101.13 or § 101.70)." 21 C.F.R. § 101.14(a)(5). The proposed health claims associate the substances, glucosamine and chondroitin sulfate, with a disease, osteoarthritis, and with disease or health-related conditions, osteoarthritis-related joint pain, joint tenderness, joint swelling, and joint degeneration and cartilage deterioration.

Osteoarthritis (also known as degenerative joint disease) is the most common form of arthritis, affecting more than 20 million Americans. Exh. 2 at 2. Osteoarthritis is a multifactorial, polygenic disorder involving mechanical, biochemical, environmental, systemic, and genetic factors that contribute to imbalance between synthesis and degradation and deterioration of cartilage matrix. Exh. 1 at 7. It is characterized by focal loss of cartilage and hypertrophic bone spurs. Exh. 1 at 7. While the term osteoarthritis refers to the overgrowth of bone in certain areas of the joint, the disease is marked by net loss of cartilage tissue. Id. Changes in the macromolecular composition of the extracellular matrix of articular cartilage are characteristic of clinically apparent osteoarthritis. Exh. 1 at 6. Those changes cause a chronic degeneration of the extracellular matrix. Id.

The primary complaint in osteoarthritis is pain, particularly concerning use of the affected joint. Id. Pain can be accompanied by varying degrees of joint stiffness, limitation of movement, tenderness and swelling at the joint margins, and loss of function. Id. Radiologically-measured decrease in joint space is significantly correlated with increase in pain severity. Id. at 8.

Each year medical care for arthritis[2] resulted in 750,000 hospitalizations and 36 million outpatient visits. CDC, Targeting Arthritis: The Nation's Leading Cause of Disability, 2003 attached as Exh. 4 at 2. In 1995, medical care for arthritis cost nearly $22 billion, and the total cost, including lost productivity, topped $82 billion, according to estimates from the American Academy of Orthopaedic Surgeons. Id. As discussed supra, the National Institutes of Health have estimated that by 2003 20 percent of Americans will be at risk for osteoarthritis. Exh. 2 at 2. Reduction in the risk of that disease; in the risk of joint pain, tenderness, and swelling associated with that disease; and in the risk of joint degeneration and cartilage deterioration is, thus, an economic and health necessity for the U.S. population.

2. Glucosamine and chondroitin sulfate contribute nutritive value at the levels present in supplements

In accordance with section 101.14(b)(3)(i), glucosamine and chondroitin sulfate contribute nutritive value. While there is no Reference Daily Intake (DRI) for either substance, the nutritive contributions of both are widely recognized. See Exhibit 1 at 12-20; see also, Exh. 3 at 93, 187 (both substances may contribute to the promotion and maintenance of the structure and function of cartilage and may also have anti-inflammatory activity).

Glucosamine is an aminomonosaccharide that serves as a substrate for the biosynthesis of chondroitin sulfate, hyaluronan, and other macromolecules located in the extracellular cartrilage matrix. Exh. 1 at 2. Chronic degeneration of the extracellular matrix of articular cartilage is a required precursor to osteoarthritis. Id. at 6. Glucosamine has immunomodulatory, anabolic, and anticatabolic properties that are a result of its interaction with intercellular and intracellular cytokine-based communication systems. Id. at 11.

[2] These statistics are for all forms of arthritis. Exh. 4(CDC) at 2. Osteoarthritis is the most common form of arthritis. Exh. 2(NIAMS) at 1

Chondroitin sulfate is a glycosaminoglycan. Id. Chondroitin sulfate polymers are secreted into the extracellular matrix of articular cartilage and are covalently bound into proteins, forming protein-polysaccharide complexes called proteoglycans. Id. at 2. Chondroitin sulfate is anticatabolic, significantly stimulates the production of proteoglycans, inhibits the destruction of articular cartilage, and directly protects articular cartilage extracellular matrix macromolecules from elevated degradative enzyme activities characteristic of asymptomatic subclinical cartilage degeneration. Id. at 11-12.

The proposed claims do not identify specific intake quantities for glucosamine or chondroitin sulfate. Studies have shown glucosamine supplementation to have nutritive value from 1500 mg to 2000 mg a day. Exh. 1 at 12-15.[3] Studies have shown chondroitin sulfate supplementation to have nutritive value from 400 mg to 1500 mg a day. Exh. 1 at 15-19. When combined, glucosamine supplementation is recommended at 1000 mg/day along with 800 mg/day of chondroitin sulfate. Id. at 21.

Glucosamine is typically supplied in solid oral dosage form in capsules containing 500 mg, 550 mg, 750 mg, and 1000 mg; liquid containing 50 mg; and tablets containing 340 mg, 500 mg, and 1000 mg. Exh. 3 at 189. Chondroitin sulfate is typically supplied in solid oral dosage form in capsules containing 250 mg, 400 mg, and 500 mg; and in tablets containing 250 mg, 400 mg, 600 mg. Id. at 96.

3. **Glucosamine and chondroitin sulfate are safe and lawful under the FDCA**

"For each such ingredient listed, the petitioner should state how the ingredient complies with the requirements of § 101.14(b)(3)(ii), e.g., that its use is generally recognized as safe (GRAS), listed as a food additive, or authorized by a prior sanction issued by the agency, and

[3] The three forms of commercially-available D-glucosamine vary in molecular size affecting their proportional weights. See Exh. 1 at 8.

what the basis is for the GRAS claim, the food additive status, or prior sanctioned status." 21 C.F.R. § 101.70(f)(A). In accordance with section 101.13(b)(3)(ii), glucosamine and chondroitin sulfate are both foods and food ingredients and are safe and lawful at the levels necessary to justify the proposed health claims.

As mentioned above, glucosamine is present in all foods containing cartilage or glycoproteins (Exh. 1 at 21) and chondroitin sulfate is present in pork byproducts and other foods. Exh. 3 at 94. The FDCA deems dietary supplements a food under 21 U.S.C. § 321(ff). Accordingly, glucosamine and chondroitin sulfate are both foods and food ingredients under 21 C.F.R. § 101.14(b)(3)(ii).

Glucosamine and chondroitin sulfate are generally recognized as safe and lawful at the levels necessary to justify the proposed health claims. General recognition of safety is based on the views of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. 21 C.F.R. § 170.30(a). The basis for such views may be either (1) scientific procedure or, in the case of a substance used in food prior to January 1, 1958, (2) through experience based on common use in food. Id.

> Safe or safety means that there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance. Safety may be determined by scientific procedures or by general recognition of safety. In determining safety, the following factors shall be considered:
> (1) the probable consumption of the substance and of any substance formed in or on food because of its use.
> (2) The cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet.
> (3) Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate.

21 C.F.R. § 170.3.

Glucosamine and chondroitin sulfate have been naturally occurring ingredients in foods consumed in the United States prior to January 1, 1958. There is no evidence that either glucosamine or chondroitin sulfate consumed in foods or as dietary supplements have a cumulative effect in the diet that affects their safety. See Exh. 1 at 21-22 (except that glucosamine may "potentiate active peptic ulcers"); Exh. 3 at 95, 188. There are no known interactions with drugs in clinical practice, except for a general warning that diabetics may need to monitor their blood glucose when taking glucosamine. Id. at 188. Moreover, there are no known harmful interactions with nutritional supplements, except for a general warning that chitosan may decrease the absorption of chondroitin sulfate. Id. at 95. Intake of glucosamine at less than 400 mg a day (for adults) is unlikely to cause adverse reactions. Exh 1 at 21. In an analysis of 16 human studies involving 372 subjects, intake of chondroitin sulfate at less than 800 mg a day did not produce more adverse events than a placebo. Exh. 1 at 22.

The PDR for Nutritional Supplements states that the most frequently reported adverse reactions associated with glucosamine are "mild gastrointestinal complaints such as heartburn, epigastric distress, and diarrhea." Exh. 1 at 21; Exh. 3 at 188. Similarly, adverse reactions associated with chondroitin sulfate are of the "mild gastrointestinal variety, such as epigastric distress, nausea, and diarrhea." Id. at 95. The PDR indicates no significant adverse effects for either substance. Exh. 3 at 95 and 188.

The maximum (safe) daily intake of both glucosamine and chondroitin sulfate is well above the amount reasonably required to accomplish the intended nutritive effect. 21 C.F.R. § 172.5. The safe upper limits for glucosamine and chondroitin sulfate have not been established. See Exh. 1 at 21-22; Exh. 3 at 95-96, 188-189. No deaths have occurred in mice and rats from glucosamine intakes of up to 5000 mg/kg body weight. Exh. 1 at 21. Dietary supplementation

with glucosamine for up to three years did not produce an increase in the incidence or severity of side effects in placebo-controlled human studies. Id. Oral supplementation in rats and rabbits with chondroitin sulfate polymers at 1g/kg body weight daily produced no effects on mutagenesis or reproductive function. Id. at 22. Dietary supplementation with chondroitin sulfate polymers for up to two years did not produce any increase in the incidence or severity of side effects in placebo-controlled human studies. Id.

The nutritive effect in reducing the risk of osteoarthritis; osteoarthritis-related joint pain, joint tenderness, and joint swelling; joint degeneration and cartilage deterioration has been recorded in daily doses ranging from 1200 mg to 2000 mg (glucosamine) and from 400 mg to 1200 mg (chondroitin sulfate) when taken separately, and from 1000 mg to 1600 mg (glucosamine) and from 800 mg to 1200 mg (chondroitin sulfate) when taken concurrently. Id. The proposed health claims thus comply with the safety and lawfullness requirements of 21 C.F.R. § 101.14(b)(3)(ii).

In summary, since glucosamine and chondroitin sulfate meet the requirements set forth in 21 C.F.R. § 101.14(b), the preliminary requirements of 21 C.F.R. § 101.70 are fully satisfied.

III. Summary of Scientific Data Supporting the Proposed Claims

A. Significant scientific agreement exists to support the proposed claims

There is significant scientific agreement among experts who study in the field of osteoarthritis that glucosamine and chondroitin sulfate are effective modifiers of the risk of osteoarthritis; the risk of osteoarthritis-related joint pain, joint tenderness, and joint swelling; and the risk of joint degeneration and cartilage deterioration. See Exh. 1 at 1. The scientific literature shows that dietary supplementation with glucosamine and chondroitin sulfate contributes to the preservation of articular cartilage, inhibits the initiation of osteoarthritic change in articular cartilage, and inhibits the progression of osteoarthritic change to symptomatic osteoarthritis. Id. at 23. Dietary supplementation with glucosamine and chondroitin sulfate is an effective modifier of osteoarthritic change and reduces the risk for osteoarthritis and of osteoarthritis-related joint pain, joint tenderness, and joint swelling. Id.

Although the mechanism of glucosamine and chondroitin sulfate's preservation and reparation effects is not entirely understood, much is known about the biochemistry and physiology of the molecules containing both substances. See Exh. 1 at 1-3, 6-12; Exh. 3 at 94, 187. Glucosamine is involved in glycoprotein metabolism. Exh. 1 at 1-2, 9-11; Exh. 3 at 187. Specifically, glucosamine appears to act by interrupting message transduction. Exh. 1 at 10. Its immunomodulatory, anabolic and anticatabolic properties result at least in part from interaction with intercellular and intracellular cytokine-based communication systems. Id. at 11.

Chondroitin sulfate appears to stimulate increases in the secretion of proteoglycans in nonosteoarthritic cartilage tissue and by embryonic articular cartilage chondrocytes in cell culture. Id. at 11. Further, chondroitin sulfate may play a role in the direct protection of articular

cartilage extracellular matrix macromolecules from the elevated degredative enzyme activities characteristic of asymptomatic subclinical cartilage degeneration. Id. at 12.

Glycoproteins, or proteoglycans, form the ground substance in the extra-cellular matrix of connective tissue. Id. The polysaccharide groups in proteoglycans are called glycosaminoglycans (GAGs) which include, among other substances, chondroitin sulfate. Id. All the GAGs contain derivatives of glucosamine. Id. GAG chains are fundamental components of aggregan found in articular cartilage, which gives that cartilage its shock-absorbing properties. Id. In later stages of joint degeneration, aggregan biosynthesis is decreased, leading to the loss of cartilage resiliency that accompanies osteoarthritis. Exh. 1 at 6-8; Exh. 3 at 187.

Within the cartilage matrix, constituents such as proteoglycans undergo a distinct turnover process during which the catabolism and removal of molecules from the extracellular matrix is in balance with the synthesis and deposition of new molecules. Exh. 1 at 1-2. A chronic imbalance in matrix macromolecule turnover producing net loss of articular tissue is a required precursor to the development of osteoarthritis and joint pain. Id. at 4. Studies have shown that glucosamine added to a culture mixture of chondrocytes harvested for osteoarthritic human articular tissue inhibited the inherent and IL-1β-induced catabolic activity of metalloproteases secreted by chondrocytes and stimulated the synthesis of physiologically-relevant proteoglycans similar to proteoglycans synthesized by chondrocytes harvested from nonosteoarthritic human articular cartilage. Id. at 10. In addition, studies have also shown that when added to a culture media of chondrocytes harvested from osteoarthritic human articular cartilage, in which adhesion of chondrocytes to fibronectin and overall protein synthesis is significantly inhibited while extracellular collagenase activity is significantly increased, glucosamine restored the adhesive properties of the chondrocytes, significantly reduced

extracellular collagenase activity and significantly increased the rate of protein syntheses. Id. at 10. Thus, glucosamine aids in the synthesis of proteoglycans and inhibits catabolic activity in the extracellular matrix of cartilage, helping to maintain the proper balance essential to cartilage health. Id. at 10-11.

Studies have shown that chondroitin sulfate stimulated significant increases in the secretion of proteoglycans in nonosteoarthritic cartilage tissue. Id. at 12. Studies with rabbits have also shown that supplementation with chondroitin sulfate significantly inhibited the destruction of articular cartilage following a collagen injection and significantly inhibited the depletion of proteoglycans in articular cartilage following subsequent injection of bradykinin. Id. at 13. Those studies suggest that chondroitin sulfate also increases the rate of protein synthesis essential to cartilage function and also suggest a role in the direct protection of articular cartilage extracellular matrix macromolecules from the elevated degradative enzyme activities characteristic of asymptomatic subclinical cartilage degeneration. Id. at 13. Human clinical trials and epidemiological studies are discussed in the following section.

B. Scientific evidence demonstrates the public health benefits of glucosamine and chondroitin sulfate

Numerous clinical trials have evaluated the effects of glucosamine and chondroitin sulfate (separately and in combination) on the risk of, and pain, tenderness, and swelling associated with, osteoarthritis, as well as the risk of joint degeneration and cartilage deterioration.

1) Glucosamine studies

In two randomized, double-blind, placebo-controlled clinical studies, compared to the effects of placebo, dietary supplementation of subjects with mild to severe femorotibial osteoarthritis with crystalline D-glucosamine sulfate for 1 month produced significantly greater

reductions in articular pain, tenderness, swelling, and restriction of movement. Exh. 1 at 13. In a recent long-term, randomized, double-blind study, 3 years of glucosamine supplementation by subjects with mild to severe femorotibial osteoarthritis produced significantly greater reductions in the mean rate of femorotibial joint space narrowing, of the WOMAC total pain score, of the WOMAC indices of total knee health, pain, function, and stiffness, of the Lequesne functional index, and of pain assessed by a visual analog scale. Id. In a far-ranging multicenter open-label study, 1208 evaluable subjects were supplemented with glucosamine for 13 to 99 days. Physician ratings of subject responses were highly favorable ("Good"- 58%; "Sufficient"-36%). Id. at 14.

Meta-analyses of available scientific studies have concluded that supplementation of glucosamine produced significantly greater reductions in the severity of osteoarthritic pain in a variety of locations, resulting in a lower level of voluntary consumption of NSAIDs (nonsteroidal anti-inflammatory drugs). Id. Numerous other studies and results are included in the scientific reported as Exh. 1. Additional studies are attached as Exhibit 7.

2) Chondroitin sulfate studies

As indicated in the enclosed scientific report and supporting science, in randomized, double-blind, placebo-controlled studies of subjects with femorotibial osteoarthritis ranging from mild to severe, dietary supplementation with chondoitin sulfate consistently produced significantly greater decreases in the Lequesne Index of functional impairment and in the severity of spontaneous joint pain assessed using a visual scale. Exh. 1 at 15. In a randomized, double-blind, placebo-controlled study of subjects with osteoarthritis of the interphalangeal joints, daily supplementation with chondroitin sulfate produced a significantly greater decrease in the number of subjects whose osteoarthritis had progressed. Id. at 16. In another randomized,

double-blind, placebo-controlled study, 2 years of dietary supplementation with chondroitin sulfate produced significantly greater decreases in the severity of spontaneous joint pain and subjects reported decreased voluntary use of NSAIDs for rescue from pain. Id. In yet another controlled study of subjects with early mild femorotibial osteoarthritis, 330 days of chondroitin sulfate supplementation produced significantly greater decreases in the severity of: spontaneous joint pain assessed using a visual analog scale, pain on passive movement, pain on active movement, pain in the evening; significantly greater increases in joint mobility and ambulation; and a significantly smaller decrease in mean articular cartilage thickness. Id. Further, in several studies, the effectiveness of chondroitin sulfate in reducing pain associated with osteoarthritis has been compared directly to the effectiveness of NSAIDs. Id. at 17.

Investigators applying meta-analyses of chondroitin sulfate supplementation studies have concluded that dietary supplementation with chondroitin sulfate for at least 120 days produced significantly greater reductions in the Lequesne Index of function impairment and in the severity of pain assessed using a visual analog scale than did placebo. Id. at 18. Other investigators concluded that dietary supplementation with chondroitin sulfate by individuals with osteoarthritis consistently produced significant decreases in joint pain and significant increases in joint function of small-to-moderate magnitude and that supplementation is "probably effective in osteoarthritis in reducing pain and in improving joint function." Id. at 18-19. Numerous other studies are analyzed in the scientific report attached as Exhibit 1 and additional studies are attached as Exhibit 7.

3) Study comparing effects of glucosamine with chondroitin sulfate

In an open label trial, subjects with femorotibial osteoarthritis consumed either glucosamine or chondroitin sulfate. After 3 months, there were no significant differences

between the groups of subjects—72% assessed their improvement as "good" without side effects. Id. at 19

4) Combination of glucosamine and chondroitin sulfate in supplementation

In one randomized, double-blind, placebo-controlled clinical trial, subjects with mild to moderate femorotibial osteoarthritis supplemented their diets with either a combination of glucosamine and chondroitin sulfate or a placebo. After 6 months, the supplementation was associated with significantly greater decreases in the Lequesne index of functional impairment. Id. at 20. In another controlled trial, dietary supplementation with a combination of glucosamine and chondroitin sulfate was compared with a placebo in subjects with painful osteoarthritis of the temporomandibular joint. Id. at 20. After 12 weeks, those supplemented with the combination exhibited significantly greater decreases in temporomandibular joint tenderness and sounds and in voluntary consumption of pain relieving medications without the production of side effects. Id. Other studies are included in the scientific report attached as Exhibit 1. Additional studies are attached as Exhibit 7.

C. Scientific summary issues

1. Is there an optimum level of glucosamine and chondroitin sulfate to be consumed beyond which no benefit would be expected?

Clinical trials have tested daily doses ranging from 1500 mg to 2000 mg (glucosamine) and 400 mg to 1200 mg (chondroitin) when taken separately, and 1000 mg to 1600 mg (glucosamine) and 800 mg to 1200 mg (chondroitin) when taken concurrently. Exh. 1 at 20-21; see also Exh. 2. The attached scientific report states that reliable and credible scientific literature indicates that daily dietary supplementation of D-glucosamine at 1000 mg/day is effective in reducing the risk of and joint pain, tenderness and swelling associated with osteoarthritis when taken with at least 800 mg/day of chondroitin sulfate. Id. at 21. There is no evidence of an

optimum level of either substance to be consumed beyond which no benefit is expected. Moreover, there are no reports of overdosage. Exh. 3 at 95, 188.

2. Is there any level at which an adverse effect from the substance or from foods containing the substance occurs for any segment of the population?

There are no levels identified at which adverse events occur for any segment of the population. Exh. 3 at 95, 188. There is a lack of reports of adverse reactions in the published scientific literature and the safety of oral supplementation with glucosamine and chondroitin sulfate have been documented in detail by several investigators. Exh. 1 at 14.

3. Are there certain populations that must receive special consideration?

The PDR for Nutritional Supplements cautions that because of insufficient safety data, pregnant women and nursing mothers should avoid using glucosamine, and those with type 2 diabetes and those who are overweight who also have problems with glucose tolerance should have their blood sugars carefully monitored. Exh. 3 at 188. The PDR also cautions that because of insufficient safety data, pregnant women and nursing mothers should avoid using chondroitin sulfate, and caution should be exercised by those taking warfarin and those with hemophilia because of "the theoretical possibility of chondroitin sulfate's antithrombotic activity." Exh. 3 at 95.

4. What other nutritional or health factors (both positive and negative) are important to consider when consuming the substance?

There are no harmful interactions with drugs for either substance. Id. at 95, 188. Glucosamine may increase insulin resistance and consequently affect glucose tolerance. Id. at 188. Thus, the PDR suggests that diabetics who decide to use glucosamine supplements will need to monitor their blood sugar and may need to adjust the dosage of the medications they take

to control blood glucose levels. Id. The PDR also states that chitosan supplementation may decrease the absorption of chondroitin sulfates if taken concurrently. Id. at 95.

D. Potential effect of the use of the proposed claims on food consumption, including significant alterations in eating habits and corresponding changes in nutrient intakes

The proposed claims may increase use of oral glucosamine and chondroitin sulfate supplements among the general population, including populations at risk of osteoarthritis. The Petitioner does not anticipate substantial dietary changes in the general population but does expect there to be some increase in consumer preferences for glucosamine and chondroitin sulfate-containing supplements. The effect on such people is expected to be beneficial, reducing the risk: of osteoarthritis; of osteoarthritis-related joint pain, tenderness and swelling; and of joint degeneration and cartilage deterioration.

E. Prevalence of the disease or health-related condition in the U.S. population and the relevance of the claims in the context of the total daily diet.

As discussed above, the proposed health claims respond to a major public health concern in the United States: osteoarthritis; osteoarthritis-related joint pain, tenderness and swelling; and joint degeneration and cartilage deterioration. Osteoarthritis is one of the most frequent causes of physical disability among adults, affecting more than 20 million Americans. National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Handout on Health: Osteoarthritis, (attached as Exhibit 2). It is the most common form of arthritis. Id. Before age 45, osteoarthritis occurs more frequently in males. After age 45, osteoarthritis occurs more frequently in females. Id. By 2030, 20 percent of Americans—about 70 million people—will have passed their 65th birthday and will be at risk for osteoarthritis. Id.

The presence of glucosamine and chondroitin sulfate in the cartilage of fish and animals has not been measured as a part of the daily diet. Supplementation is the most effective method

to achieve the levels of glucosamine and chondroitin sulfate in order to have nutritive effect. As the attached scientific report indicates, the reliable and credible scientific literature indicates that daily dietary supplementation with 1500 mg of D-glucosamine sulfate is effective in reducing the risk of, and joint pain, tenderness and swelling associated with, osteoarthritis when taken alone, and 1000 mg a day when combined with chondroitin sulfate. Id. at 21. The reliable and credible scientific literature indicates that daily dietary supplementation with 1200 mg of chondroitin sulfate, consumed alone or with glucosamine is effective in reducing the risk of osteoarthritis and of joint pain, tenderness, and swelling associated with osteoarthritis. Id. Thus, both substances offer a safe, inexpensive, and readily accessible means for reducing the risk of osteoarthritis; osteoarthritis-related joint pain, tenderness, and swelling; and joint degeneration and cartilage deterioration.

IV. Analytical Method

The amount of glucosamine contained in a dietary supplement that may be a candidate for bearing the health claims can be ascertained by High-Performance Liquid Chromatography (HPLC) according to the Petitioner, Weider Nutrition International, the Institute for Nutraceutical Advancement, and the University of Alberta, Edmonton, Alberta, Canada, as well as by Glucosamine Rapid Assay, according to the Department of Microbiology and Immunology, University of British Columbia. See Exh. 5. The amount of chondroitin sulfate contained in a dietary supplement that may be a candidate for bearing the health claims can be ascertained by the Cetylpyridinium Chloride (CPC) Titration Method according to the Petitioner and the Institute for Nutraceutical Advancement. See Exh. 6.

V. Proposed Model Claims

Petitioner proposes the follow model claims for glucosamine and chondroitin sulfate:

- **Glucosamine may reduce the risk of osteoarthritis.**
- **Chondroitin sulfate may reduce the risk of osteoarthritis.**
- **Glucosamine and chondroitin sulfate may reduce the risk of osteoarthritis.**
- **Glucosamine may reduce the risk of osteoarthritis-related joint pain, tenderness and swelling.**
- **Chondroitin sulfate may reduce the risk of osteoarthritis-related joint pain, tenderness and swelling.**
- **Glucosamine and chondroitin sulfate may reduce the risk of osteoarthritis-related joint pain, tenderness and swelling.**
- **Glucosamine may reduce the risk of joint degeneration.**
- **Chondroitin sulfate may reduce the risk of joint degeneration.**
- **Glucosamine and chondroitin sulfate may reduce the risk of joint degeneration.**
- **Glucosamine may reduce the risk of cartilage deterioration.**
- **Chondroitin sulfate may reduce the risk of cartilage deterioration.**
- **Glucosamine and chondroitin sulfate may reduce the risk of cartilage deterioration.**

Multiple studies have shown that oral supplementation with glucosamine and chondroitin sulfate significantly reduces the risk of osteoarthritis; osteoarthritis-related pain, tenderness, and swelling; joint degeneration; and cartilage deterioration. Moreover, clinical trials have proven the safety of those substances for the general population.

VI. Attachments

Attached are copies of the scientific studies and other information referenced in, and constituting the basis for, this petition. To the best of the Petitioner's knowledge, all non-clinical studies relied upon were conducted in compliance with the good laboratory practices regulations set forth in 21 C.F.R. Part 58, and all clinical or other human investigations relied upon were either conducted in accordance with the requirements for institutional review set forth at 21

C.F.R. Part 56 or were not subject to such requirements in accordance with 21 C.F.R. § 56.104 or 56.105, and were conducted in conformance with the requirements for informed consent set forth in 21 C.F.R. § 50 eq seq. See generally, 21 C.F.R. § 101.7 (c)-(d).

VII. Environmental Impact

The requested health claim approvals sought in this petition are categorically excluded from the environmental impact statement requirements under 21 C.F.R. § 25.24.

VIII. Conclusion and Certification

For the foregoing reasons, the Petitioner requests that the FDA approve the proposed health claims. The Petitioner looks forward to working with the FDA in promulgating a regulation authorizing the use of dietary supplement health claims concerning the association between glucosamine and chondroitin sulfate and the risk of osteoarthritis; of osteoarthritis-related pain, tenderness, and swelling; and of joint degeneration and cartilage deterioration.

Any questions concerning this Petition may be directed to Jonathan W. Emord, Esq. or to Claudia A. Lewis-Eng, Esq., Emord & Associates, P.C., 5282 Lyngate Court, Burke, VA 22015 (202) 466-6937.

The undersigned certify on behalf of the Petitioner that to the best of Petitioner's knowledge and belief, the Petition includes all information and views on which the Petitioner relies and is a representative and balanced submission that includes unfavorable information as

well as favorable information known by the Petitioner to be pertinent to evaluation of the proposed health claims.

Sincerely,

Jonathan W. Emord

Claudia A. Lewis-Eng

Andrea G. Ferrenz
Jonathan R. Goodman
Kathryn E. Balmford

Counsel to

Weider Nutrition International, Inc.

Emord & Associates, P.C.
5282 Lyngate Court
Burke, VA 22015
P: (202) 466-6937
F: (202) 466-4938
Email: jemord@emord.com; clewis-eng@emord.com
Date submitted: May 29, 2003

EXHIBIT 1

Based on my review of the reliable and credible scientific literature regarding articular cartilage biochemistry and physiology, cartilage degeneration, degenerative joint disease and osteoarthritis, I conclude that there is significant scientific agreement in support of the following health claims:

- Glucosamine may reduce the risk of osteoarthritis.
- Chondroitin sulfate may reduce the risk of osteoarthritis.
- Glucosamine and chondroitin sulfate may reduce the risk of osteoarthritis.
- Glucosamine may reduce the risk of osteoarthritis-related joint pain, tenderness and swelling.
- Chondroitin sulfate may reduce the risk of osteoarthritis-related joint pain, tenderness and swelling.
- Glucosamine and chondroitin sulfate may reduce the risk of osteoarthritis-related joint pain, tenderness and swelling.
- Glucosamine may reduce the risk of joint degeneration.
- Chondroitin sulfate may reduce the risk of joint degeneration.
- Glucosamine and chondroitin sulfate may reduce the risk of joint degeneration,
- Glucosamine may reduce the risk of cartilage deterioration.
- Chondroitin sulfate may reduce the risk of cartilage deterioration.
- Glucosamine and chondroitin sulfate may reduce the risk of cartilage deterioration.

Composition and Physiologic Functions of Articular Cartilage and the Biochemical and Physiologic Roles of D-Glucosamine and Chondroitin Sulfate

Cartilage is composed of a complex extracellular matrix of collagen and elastic fibers within a hydrated gel of glycosaminoglycans and proteoglycans. This specialized network is stabilized by means of intermolecular and intramolecular cross-links that harness the swelling pressure exerted by the high concentration of negatively charged aggregates.[1] This accounts for more than 98% of the articular cartilage volume; cellular components constitute the remaining 2%. The interaction of these matrix components imparts the characteristic biomechanical properties of flexibility and resistance to compression. The collagen component of the cartilage matrix is relatively inert, but the other constituents, such as proteoglycans, undergo a distinct turnover process during

which the catabolism and removal of molecules from the extracellular matrix is in balance with the synthesis and deposition of new molecules.[2]

Proteoglycans are large macromolecules consisting of multiple chains of glycosaminoglycan disaccharides and oligosaccharides attached to a central protein core that provide a framework for collagen and also bind water and cations, forming a viscous, elastic layer that lubricates and protects the cartilage tissue. The presence of these negatively charged aggregates imparts to the matrix of articular cartilage its strong affinity for water and is the most significant contributor to the biomechanical properties of cartilage. The glycosaminoglycans most common in human connective tissue include the disaccharides keratan sulfate, dermatan sulfate, heparin sulfate and chondroitin sulfate and the oligosaccharide, hyaluronan. They consist of amino sugars, which are repeating disaccharide units composed of a hexuronic acid (D-glucuronic acid, iduronic acid, or L-galactose) and a hexosamine (D-glucosamine or D-galactosamine).[3,4]

The main disaccharide units of cartilage glycosaminoglycans are formed by the (1→3) linkage of D-glucuronic acid to *N*-acetylglucosamine; disaccharide units are linked by β(1→4) galactosamine links. The D-galactosamine residues are sulfated either in position 4 (as in chondroitin-4-sulfate) or 6 (as in chondroitin-6-sulfate). The sulfate groups, together with the carboxyl groups of D-glucuronic acid, are ionized at tissue pH, conferring to the chain a strong global electronegative charge.[5-10] Inadequate sulfate availability resulting in the production of undersulfated proteoglycans will reduce their electronegative charge and water carrying capacity.[11,12]

Glucosamine (2-amino-2-deoxyalpha-D-glucose) is an aminomonosaccharide that serves as a substrate for the biosynthesis of chondroitin sulfate, hyaluronan, and other macromolecules located in the extracellular cartilage matrix. The conversion of L-glutamine and D-fructose-6-P to L-glutamate and D-glucosamine by L-glutamine-D-fructose-6-P amidotransferase (E.C. 2.6.1.16) is the rate-limiting step in proteoglycan synthesis.[13-15] This reaction may be bypassed if D-glucosamine is available within the cell cytoplasm.[16,17] Whatever its source, D-glucosamine is phosphorylated and the resulting D-glucosamine-6-P is acetylated to *N*-acetyl- D-glucosamine, the common precursor for the biosynthesis of keratan sulfate, dermatan sulfate, chondroitin sulfate and hyaluronan.[16,17]

Chondroitin sulfate is a glycosaminoglycan that is polymerized into long, unbranched polysaccharide chains in which some of the constituent chondroitin moieties (composed of D-glucuronic acid and *N*-acetyl- D-glucosamine) are sulfated.[18] Close control of chondroitin sulfate synthesis determines chain length, disaccharide composition and degree of sulfation, which vary with anatomic location, stage of development and age and are heterogeneous.[19-24] For example, the sulfation pattern of chondroitin disaccharides in normal human articular cartilage varies. The deeper layers of immature cartilage contain 4 times more sulfated residues than the upper regions of the immature tissue contain (as a result of polysulfation of some chondroitin residues in the extracellular matrix of the deeper regions).[19-21] All regions of the extracellular matrix of immature articular

cartilage contain a smaller ratio of chondroitin-6-sulfate to chondroitin-4-sulfate than is typical of the extracellular matrix of articular cartilage in adults.[19-21]

Chondroitin sulfate polymers are secreted into the extracellular matrix covalently bound to proteins, forming protein-polysaccharide complexes called proteoglycans. In a proteoglycan, about 100 chondroitin sulfate chains, each containing 50 to 60 disaccharide units of chondroitin sulfate, are covalently attached to a polypeptide backbone composed of over 2,000 amino acids (the serine-rich core protein with a molecular weight of 250,000 to 300,000 daltons). This covalent *O*-linkage occurs between a terminal D-xylose or D-galactose residue that had been added to the polysaccharide chain and a serine or threonine residue on the core protein, with one chondroitin sulfate chain per 20 or so amino acid residues. The total molecular weight of an individual proteoglycan monomer is 1,500,000 to 2,500,000 daltons.[5]

One end of the core protein of a proteoglycan is non-covalently linked to a long polysaccharide filament of hyaluronan through a link protein; the connection is achieved by a globular region of the link protein that surrounds the terminal portion of the core protein and a stretch of 5 disaccharide units along the length of the hyaluronan chain.[25,26] There are two structurally related N-terminal globular domains, G1 and G2, of which only G1 (and not G2) is involved in the aggregation of proteoglycans with hyaluronan. The interglobular domain joining G1 and G2 contains proteinase-sensitive sequences which appear to be the key sites for cleavage during aggrecan turnover.[5] Approximately 100 core proteins are bound to an individual hyaluronan chain, at regular intervals of 300 A, forming a unit of aggrecan, the large molecular mass proteoglycan-hyaluronan aggregate predominant within the extracellular matrix of articular cartilage.

The hydrodynamic properties of this aggregate determine the load-bearing capacity of articular tissue. As the electronegative charges of aggrecan draw water into the tissue, a large osmotic swelling pressure is created that swells and expands the extracellular matrix. This pressure produces tension within the interlacing collagen network of the matrix; balance is achieved when tension in the collagen network prevents further entry of water. Articular cartilage tissue swollen with water expresses substantial compressive resilience and offers considerable resistance to fluid flow and redistribution of water. Fully hydrated articular cartilage tissue behaves as a stiff elastic polymer when exposed to sudden impact loading, with pressure-induced displacement of water from the matrix with little or no effect on matrix macromolecules (although sustained loads will produce slow inelastic deformation). Removal of loading allows re-entry of water and a return to the pre-loading high-tension equilibrium condition.[5,18,27-29]

Age and the Composition of Articular Cartilage

In rabbits, fetal articular cartilage is softer than is adult articular cartilage because fetal articular cartilage contains a greater proportion of polysulfated chondroitin sulfates and therefore its water binding capacity is greater.[30] In rats, as age increases from birth to

mature adulthood, the extent to which nonosteoarthritic articular cartilage extracellular matrix chondroitins are sulfated decreases significantly.[31] In dogs, increasing age is accompanied by significantly decreased chondroitin sulfate and proteoglycan content of articular cartilage and reduced aggregability of the remaining proteoglycans.[32] Similarly, calf articular cartilage proteoglycans are larger on average than are proteoglycans in nonosteoarthritic adult bovine articular cartilage (and contain larger chondroitin sulfate polymers).[33] In addition to decreasing average size of matrix proteoglycans and chondroitin polymers, the ratio of chondroitin 6-sulfate to chondroitin 4-sulfate in the extracellular matrix of articular cartilage increases with increasing age.[34]

In humans, increasing age is accompanied by a decreasing proportion of chondroitin sulfates in the extracellular matrix of nonosteoarthritic articular cartilage[35] and increases in the ratio of chondroitin 6-sulfate to chondroitin 4-sulfate[36,37] and in the free glucosamine content of the tissue.[38] Furthermore, the average chondroitin sulfate content of individual articular cartilage proteoglycans decreases, impairing the ability of proteoglycans to aggregate spontaneously with hyaluronan.[39] In addition, the ability of proteoglycans to aggregate spontaneously with hyaluronan is decreased as a result of an increased incidence of defect in the core protein of newly-synthesized proteoglycans.[40] Consequently, the aggrecan content of the extracellular matrix of articular cartilage in adults is significantly lower than that in children.[40]

In "normal but aged" human chondrocytes (mean age of donor: 68.8 +/- 4.2 years), basal (unstimulated) synthesis of matrix-degrading stromelysin-1 and collagenase is significantly greater than in chondrocytes harvested from joints of "normal young adults" (mean age of donor: 28.6 +/- 7.1 years). Therefore, "aging" may sensitize chondrocytes to the effects of accelerators of extracellular matrix degradation and may increase the requirement of chondrocytes for exogenous substrate to support the synthesis of new and replacement matrix macromolecules.[41]

Precipitating Events Producing Cartilage Degeneration and Mechanical Failure

Osteoarthritis is a multifactorial, polygenic disorder involving mechanical, biochemical, environmental, systemic and genetic factors that contribute to imbalance between synthesis and degradation of cartilage matrix.[42,43] Chronic imbalance in matrix macromolecule turnover producing net loss of articular tissue is a required precursor to the development of osteoarthritis and joint pain.

There are numerous potential etiologic triggers that can initiate the progression of events culminating in tissue failure. For example, quadriceps muscle weakness significantly increases the risk for osteoarthritis in humans[44] and laxity in a joint may precede failure of the cartilage matrix.[45] Interstitial fluid pressurization during loading contributes more than 90% of load support, shielding the collagen-proteoglycan matrix from excessive stresses and reducing friction at the articular surfaces.[46] A chronic imbalance of shock-

absorbing and weight–bearing muscles affecting joint alignment[47,48] or overloading from excessive body weight[49] induces a mild yet chronic metabolic imbalance in the affected articular cartilage.

Whenever mechanical stress exceeds the tissue's load-bearing capacity, chondrocyte and synoviocyte secretion of the cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) and nitric oxide (NO) is stimulated. These cytokines auto-stimulate chondrocyte and synoviocyte secretion of matrix metalloproteinases (collagenase, gelatinase, aggrecanase, elastase, and fibronectin-degrading stromelysin-1) and inhibit chondrocyte synthesis of cartilage-specific proteoglycans and type II collagen. The resulting imbalance between synthesis and degradation of extracellular matrix components results in a net decrease in matrix content of aggrecan, type II collagen and other matrix macromolecules.[42,43,50]

IL-1β, IL-6, TNF-α and nitric oxide also stimulate the clonal expansion of chondrocytes whose daughter cells may express a "fetal" differentiation pattern during early metabolic imbalance in articular cartilage[51] and produce inferior repair matrix prone to fibrillation and mechanical failure.[42,43] Spontaneous repair matrix produced in early asymptomatic subclinical osteoarthritic change exhibits a heterogeneous composition more closely resembling that of fibrous cartilage,[52] with inferior biomechanical competence[53,54] resulting in functional incompetence and perishability.[55] In addition, the abnormal newly-synthesized matrix may be fibronectin-deficient or may undergo accelerated hydrolysis of fibronectin by stromelysin-1, in either case disturbing chondrocyte anchorage to the extracellular matrix ("anchorage dependence") and inducing apoptosis and hypocellularity (chondrocyte survival requires attachment to substrate).[42,43]

In early asymptomatic subclinical osteoarthritic change in humans, reactive proliferation of extracellular articular cartilage matrix results in the production of abnormally large and more extensively sulfated chondroitin sulfate polymers and significantly decreased total glycosaminoglycan content (similar to the matrix of nonosteoarthritic human articular cartilage after partial enzymatic hydrolysis[56]) and significantly decreased proportion of proteoglycans of nonosteoarthritic molecular sizes.[57-60] Overall, there is a significantly increased proportion of nonaggregated proteoglycans, significantly decreased average size of proteoglycan aggregates (aggrecan) and incorporation of significantly smaller-than-normal-for-age chondroitin sulfate chains into newly-synthesized proteoglycans, significantly decreased total chondroitin sulfate content (and therefore decreased water binding capacity), and a significantly lower ratio of chondroitin 6-sulfate to chondroitin 4-sulfate.[21,61-63] Both the abnormally small proteoglycans and the abnormally large proteoglycans are unable to aggregate with hyaluronan to form aggrecan.[64] In addition, osteoarthritic human articular cartilage exhibits increased synthesis of more readily hydrolyzable (easily degradable) collagens.[65,66]

In cell culture, human articular chondrocytes harvested from osteoarthritic joint cartilage produced proteoglycans that differed from those produced by human articular

chondrocytes harvested from nonosteoarthritic joint cartilage.[67] These proteoglycans resembled "fetal-type" proteoglycans with increased chondroitin 4-sulfate content and an increased percentage of smaller proteoglycans than is typical of the proteoglycans produced by chondrocytes harvested by nonosteoarthritic human articular cartilage.[68] The synthesis of temporally inappropriate proteoglycans is accompanied by a significantly accelerated rate of degradation of older, more typical-for-age proteoglycans.[69]

In a rat model of the initiation of osteoarthritic change, increased mechanical stress on articular cartilage increases the ratio of chondroitin 6-sulfate to chondroitin 4-sulfate in the extracellular matrix.[70] Mechanical compression of articular cartilage stimulates intrachondrocytic cyclo-oxygenase activity, resulting in increased production of PGE_2, an inducer of inducible NO synthase-2 (iNOS) activity; consequently, intrachondrocytic NO production is increased in proportion to the magnitude of compression and increasing local compression increases the recruitment of compression-responsive NO-producing articular chondrocytes.[71] NO stimulates chondrocytic synthesis of matrix metalloproteinases,[72] nascent (inactive) IL-1β,[73-75] and interleukin-1-converting enzyme (ICE).[76] ICE activates nascent inactive IL-1β.[75] Activated IL-1β inhibits chondrocytic synthesis of proteoglycans[73,74] and collagen[73,74] and stimulates chondrocytic synthesis of stromelysin-1,[41] collagenase[41] and a presumptive aggrecanase enzyme that cleaves aggrecan.[77] As osteoarthritic change progresses, IL-1β also stimulates increased NO production;[78-81] NO further stimulates chondrocytic synthesis of matrix metalloproteinases[72] and accelerates the progression of osteoarthritis through the establishment of a cooperative positive feedback cycle.[82,83] In addition, chondrocytes harvested from osteoarthritic human articular cartilage synthesize growth-related oncogene-α (GRO-α) in response to IL-1β; GRO-α stimulates degradation of fibronectin by stromelysin-1, producing anoikis (cell death resulting from loss of normal cell-substratum contact).[84]

Chondrocytes harvested from osteoarthritic human joints exhibit a reduced anabolic response to insulin-like growth factor-1 (IGF-1) ("IGF resistance"[42,85]) and may have reduced ability to transport glucose from the extracellular fluid into the cell for glycosaminoglycan synthesis.[86] Therefore, osteoarthritic chondrocytes may have an increased requirement for glucosamine of extracellular origin.[42,87-89] In addition, IGF-1 stimulates net synthesis of proteoglycans able to form aggrecan by nonosteoarthritic adult bovine articular chondrocytes in cell culture.[90] "IGF resistance" may contribute to the etiology of osteoarthritis by down-regulating the production of replacement aggrecan.

Oxidative stress also may impair the synthesis of matrix macromolecules by articular chondrocytes. Inhibition of chondrocyte γ-glutamyl-cysteine synthetase results in reduced intrachondrocytic glutathione concentration and decreased incorporation of sulfate into newly-synthesized proteoglycans and of proline into newly-synthesized collagen.[91]

Chronic Degeneration of the Extracellular Matrix of Articular Cartilage is a Required Precursor to Osteoarthritis

Changes in the macromolecular composition of the extracellular matrix of articular cartilage are characteristic of clinically apparent osteoarthritis. The ratio of chondroitin 6-sulfate to chondroitin 4-sulfate in the extracellular matrix of the articular cartilage of osteoarthritic mice is significantly greater than the ratio in the extracellular matrix of articular cartilage in age-matched nonosteoarthritic mice.[92] Osteoarthritic rat articular cartilage, compared to nonosteoarthritic articular cartilage, exhibits significantly decreased total proteoglycan, chondroitin 4-sulfate and chondoitin 6-sulfate contents and significantly increased stromelysin-1 (fibronectin-degrading) activity.[93] In addition, the percentage of apoptotic chondrocytes in the tissue is significantly increased.[93] Proteoglycans in osteoarthritic adult bovine articular cartilage are larger than normal adult bovine articular cartilage proteoglycans (with larger chondroitin sulfate polymers) and closely resemble proteoglycans found in the articular cartilage matrix of calves.[33] Osteoarthritic equine articular cartilage contains a significantly increased proportion of unsulfated disaccharides and a significantly decreased proportion of chondroitin 6-sulfate.[94] The articular cartilage of Cynomolgus macaque monkeys with arthritis exhibits increased production of abnormal chondroitin sulfate-containing polymers.[95]

In degenerative joint disease in dogs, affected articular cartilage contains significantly increased amounts of newly-synthesized large chondroitin sulfate-rich and glucosamine- and galactosamine-poor proteoglycans typical of those produced by immature canine articular cartilage.[61,96,97] As cartilage degeneration progresses, affected canine articular cartilage exhibits significantly increased production of abnormal chondroitin sulfate-containing polymers, significantly increased water content, significantly increased proteoglycan content, significantly increased percentage of smaller proteoglycans and significantly decreased percentage of chondroitin sulfate in proteoglycans.[57,98,99] Some newly synthesized proteoglycans are abnormally large (containing abnormally long chondroitin sulfate chains) and a second population of proteoglycans are abnormally small; both have lost the ability to aggregate spontaneously with hyaluronan, compromising the hydrodynamic properties of the tissue.[100]

Pathologic changes in cartilage matrix composition and organization alter the affinity of the matrix for water and produce excessive cartilage deformation under loading.[101,102] When chronic, excessive tissue deformation induces adaptive structural and compositional changes that confer increased stiffness in the tissue,[45] increasing its vulnerability to the compressive, tensile and shear forces that occur during normal joint function.[18] Grossly apparent cartilage erosion does not appear until the tissue has lost considerable stiffness and is undergoing progressive mechanical failure.[45]

As a result of the changes occurring in articular cartilage, abnormally transmitted mechanical stress produces microfractures within the tissue matrix that in turn increase the stresses on surrounding tissue and induce increased chondrocyte secretion of metalloproteinases.[103] The subsequent enzymatic tissue degradation potentiates local tissue stress and initiates a positive feedback loop. Increased loading on subchondral bone stimulates the attempt to reduce mechanical stress by increasing joint surface area through the production of bone spurs (osteophytes) at the joint margins (which confer the hard bony enlargement that is characteristic of chronic osteoarthritis).[103]

The Culmination of Matrix Degeneration in Osteoarthritis

In the US, the incidence of at least one joint with osteoarthritis among those aged 15 to 40 years is about 5%; this increases to over 60% among those over 65 years old.[104] Overall, the prevalence of at least mildly symptomatic osteoarthritis in at least one joint is about 30%.[105] Symptomatic osteoarthritis of the knee occurs in about 6% of US adults aged 30 years and older,[106] although radiographic changes of the femorotibial compartment occur in 5% to 15% of people aged 35 to 74 years.[107]

Clinical osteoarthritis (also known as degenerative joint disease) is characterized by focal loss of cartilage and hypertrophic bone spurs.[103] Although the term osteoarthritis refers to the overgrowth of bone at the margins and subchondral areas of the joint, and despite the eventual bony involvement in later stages of the disease, osteoarthritis is marked by net loss of cartilage tissue. Initial loss of articular cartilage tissue is mild but may progress to full thickness erosions and eventual bone-to-bone contact (loss of all joint space). Narrowing of the joint space may reflect other degenerative changes in addition to articular cartilage erosion;[108] as cartilage degeneration progresses, subchondral bone density and volume increase (consistent with increased transmission of load bearing into the subchondral bone).[109]

The primary complaint in osteoarthritis is pain, particularly upon use of the affected joint.[103] Pain can be accompanied by varying degrees of joint stiffness, limitation of movement, tenderness and swelling at the joint margins and loss of function. Osteoarthritis often is asymmetric. There are no systemic symptoms outside the affected joint.[103]

Possible causes of pain in human osteoarthritis include osteophyte growth with stretching of the periosteum, increased intraosseous pressure, microfractures, ligament damage, capsular tension, meniscal injury and synovitis.[110] Radiologically measured decrease in joint space is significantly correlated with increase in pain severity, although the clinical utility of pain assessment as an estimator of joint deterioration is under debate.[111]

Bioavailability of Supplemental Glucosamine and Chondroitin Sulfate

D-Glucosamine: There are 3 forms of commercially-available D-glucosamine: D-glucosamine (MW: 179), D-glucosamine-HCl (MW: 270) and D-glucosamine sulfate (a derivative of the naturally occurring cartilage extracellular matrix constituent, aminomonosaccharide D-glucosamine;[112] MW: 456). Because of the differences in molecular size, 1500 mg of D-glucosamine-HCl provides as much D-glucosamine as is provided by 2600 mg of D-glucosamine sulfate or 1040 mg of D-glucosamine. A daily intake of 1500 mg of D-glucosamine sulfate is equivalent to a daily intake of between 15 and 30 mg/kg body weight.

In studies in rats, 90% to 95% of ingested D-glucosamine sulfate was absorbed intact into the blood and about 30% of newly absorbed D-glucosamine sulfate was incorporated into newly synthesized proteoglycans in articular cartilage tissues.[113,114] In studies in humans, consumption of 314 mg of crystalline D-glucosamine sulfate was followed by the absorption of about 280 mg (about 90%) intact into the bloodstream; about 50% of this amount (about 140 mg) survived hepatic first-pass extraction intact.[115] When the consumption of 1884 mg occurred as one bolus or in three divided intakes of 626 mg every 4 hours, there was no difference in total D-glucosamine sulfate bioavailability to systemic tissues (about 40% to 50% of the amount ingested). Other investigators have reported that over 90% of ingested D-glucosamine sulfate was absorbed intact into the human enterohepatic circulation.[116,117] One investigator reported that about 75% of ingested D-glucosamine sulfate was bioavailable to body tissues following hepatic first-pass extraction.[117]

In healthy subjects, ingestion of D-glucosamine sulfate was followed by increased serum sulfate concentration. In contrast, ingestion of sodium sulfate did not effect serum sulfate concentration, suggesting that dietary supplementation with D-glucosamine sulfate might provide D-glucosamine, free sulfate and D-glucosamine sulfate for proteoglycan synthesis.[118]

Chondroitin sulfate: In dogs, rats, mice and rabbits, about 0% to 15% of an ingested mix of chondroitin sulfates was absorbed intact.[42,119-124] In these species, absorption favors chondroitin sulfate polymers with molecular weights <14,000 daltons.[120] In all species studied, some inorganic SO_4^{-2} also was absorbed following cleavage of SO_4^{-2} from the chondroitin sulfate polymers by sulfatases.[42,119-124]

In humans, between 0% and 15% of an oral bolus of chondroitin sulfates is absorbed intact into the blood.[125-128] In addition, another 10% to 20% is absorbed following hydrolysis to smaller polymers (<5000 daltons) prior to absorption.[128,129] However, the biological activity of these smaller polymers has been questioned.[17] The absorption of chondroitin sulfates probably is not nil; the consumption of either 800 mg or 3000 mg of mixed chondroitin sulfates significantly increased plasma chondroitin sulfate concentration 3 hours after ingestion[130,131] and the consumption of 800 mg daily for 5

days increased plasma chondroitin sulfate concentration from undetectable levels to a mean of 1.80 mcg/mL, suggesting that the systemic bioavailability of intact chondroitin sulfates in humans is about 12% of the amount ingested.[131]

Biochemical and Physiologic Roles of D-Glucosamine in the Preservation of Articular Cartilage

In cultures of chondrocytes harvested from nonosteoarthritic rat articular cartilage, IL-1β inhibits the expression of UDP-glucoronosyltransferase I mRNA, resulting in decreased synthesis of proteoglycans and their precursors.[132,133] Conversely, IL-1β stimulates intrachondrocytic production of the catabolism-inducing factors, NO and PGE_2, resulting in increased expression of mRNA coding for the extracellular fibronectin degrading metalloproteinase enzyme, stromelysin-1.[132] The addition of D-glucosamine to the culture medium prevented IL-1β-induced inhibition of the expression of UDP-glucuronosyltransferase I mRNA[132,133] and of proteoglycan synthesis,[132,133] as well as IL-1β-induced activation of pro-apoptotic nuclear factor KB (NF-KB).[133] The addition of D-glucosamine-HCl to the culture medium of nonosteoarthritic equine articular cartilage explants in organ culture prevented IL-1β-induced increases in the activities of stromelysin-1, collagenase and gelatinase and bacterial lipopolysaccharide (LPS)- and IL-1β-induced increases in the production of NO and PGE_2 and the degradation of extracellular matrix proteoglycans.[134-137] Similarly, crystalline D-glucosamine sulfate added to the culture media of chondrocytes harvested from osteoarthritic human articular cartilage inhibited the inherent[138,139] and IL-1β-induced[132,139] catabolic activity of metalloproteases secreted by the chondrocytes and stimulated the synthesis of physiologically-relevant proteoglycans with chemical characteristics of proteoglycans synthesized by chondrocytes harvested from nonosteoarthritic human articular cartilage.[138,140] By unknown but presumably similar mechanisms, dietary supplementation with D-glucosamine sulfate (50 mg/kg body weight daily) conferred to rats resistance to kaolin-and adjuvant-induced tibio-tarsal arthritis.[141]

Both D-glucosamine-HCl and D-glucosamine sulfate added to the culture medium of nonosteoarthritic rat femoral articular cartilage explants in organ culture significantly increased the rates of collagen and proteoglycan synthesis and partially prevented nonsteroidal anti-inflammatory drug- (NSAID)-induced inhibition of proteoglycan synthesis.[14] Similarly, crystalline D-glucosamine sulfate stimulated the production of proteoglycans by chondrocytes harvested from nonosteoarthritic human articular cartilage in cell culture.[142] When added to the culture media of chondrocytes harvested from osteoarthritic human articular cartilage, in which adhesion of chondrocytes to fibronectin and overall protein synthesis are significantly inhibited while extracellular collagenase activity is significantly increased, D-glucosamine restored the adhesive properties of the chondrocytes,[143] significantly reduced extracellular collagenase activity[138] and significantly increased the rate of protein synthesis.[138] Osteoarthritic articular cartilage tissue samples harvested from rabbits that had been fed diets supplemented with D-

glucosamine-HCl (20 mg/kg body weight daily) exhibited significantly accelerated rates of synthesis of new proteoglycans compared to articular cartilage tissue samples harvested from unsupplemented animals.[144]

D-glucosamine appears to act by interrupting message transduction. Following transport across the chondrocyte cell membrane by the GLUT-2 and GLUT-4 glucose transporters,[145,146] supplemental D-glucosamine stimulated the expression of IL-1 cell membrane receptor subtype II, which binds IL-1β with high affinity but produces an inactive receptor-ligand complex, effectively intercepting IL-1β-based signal transmission.[133] In addition, when D-glucosamine was added to the culture medium of nonosteoarthritic bovine articular cartilage explants in organ culture in concentrations that significantly inhibited IL-1β-induced aggrecanase cleavage of aggrecan, lactate production was unaffected and D-glucosamine was incorporated into newly-synthesized chondroitin sulfates.[113] D-Glucosamine-HCl also stimulated sulfate incorporation into chondroitin sulfates in the extracellular matrix of nonosteoarthritic bovine articular cartilage explants in organ culture.[147] In contrast, concentrations of D-glucosamine-HCl sufficiently high to compromise cell viability in nonosteoarthritic bovine articular cartilage explants in organ culture[148] or in nonosteoarthritic canine chondrocytes in cell culture[149] also significantly inhibited proteoglycan synthesis. These findings indicate that the inhibition of IL-1β-induced catabolism was not an artefact of D-glucosamine-induced general inhibition of chondrocyte cellular metabolism.[139]

In other cell culture models, D-glucosamine-HCl (0.01 to 1.0 mM) dose-dependently suppressed the superoxide anion generation induced by formyl-Met-Leu-Phe (fMLP) or complement-opsonized zymosan and inhibited the phagocytosis of complement-opsonized zymosan or IgG-opsonized latex particles.[150] Similarly, D-glucosamine-HCl significantly inhibited fMLP-induced up-regulation of CD11b, polymerization of actin, and activation via phosphorylation of pro-apoptotic p38 mitogen-activated protein kinase (MAPK).[150] In addition, D-glucosamine-HCl inhibited the release of lysozymes from phagocytozing neutrophils and suppressed neutrophil chemotaxis toward zymosan-activated serum.[150] Furthermore, supplemental D-glucosamine inhibited the activation of T-lymphocytes and the reactivity of leukocytes without producing signs of cellular toxicity.[151] All of the effects of supplemental D-glucosamine provide evidence that its immunomodulatory, anabolic and anticatabolic properties result at least in part from interaction with intercellular and intracellular cytokine-based communication systems.

Biochemical and Physiologic Roles of Chondroitin Sulfate in the Preservation of Articular Cartilage

In cultures of chondrocytes harvested from nonosteoarthritic human knee joint articular cartilage, supplemental crystalline chondroitin sulfate polymers (Condrosulf®, Sanova Pharma, Vienna, Austria; 55% chondroitin 4-sulfate, 38% chondroitin 6-sulfate, 5% unsulfated chondroitin sulfates, 1% disulfated chondroitin sulfates, 1% non-chondroitin

compounds, average molecular weight: 24,000 daltons) bind to a specific cell membrane receptor, possibly CD36, prior to transport into the chondrocyte cell cytoplasm.[152] When added to culture media, both Condrosulf® and a synthetic mixture of chondroitin 4-sulfate and chondroitin 6-sulfate (Structum®, Smith Kline Corp. Philadelphia, PA; undefined polydisperse mixture of chondroitin 4-sulfate and chondroitin 6-sulfate) significantly stimulated the production of proteoglycans by nonosteoarthritic human articular cartilage chondrocytes in cell culture.[142, 153] Paradoxically, high concentrations (>1000 mcg/mL) of Condrosulf® in the culture medium induced concentration-dependent downregulation of the expression of mRNA coding for aggrecan.[154]

An undefined mix of chondroitin sulfates stimulated significant increases in the secretion of proteoglycans in nonosteoarthritic cartilage tissue[155] and by embryonic articular cartilage chondrocytes in cell culture.[156] Chondroitin 4-sulfate alone added to the culture medium stimulated significant increases in the secretion of proteoglycans and in the incorporation of sulfate into chondroitin-containing proteoglycans by embryonic articular cartilage chondrocytes in cell culture.[157]

A synthetic mixture of chondroitin 4-sulfate and chondroitin 6-sulfate (Structum®) prevented IL-1β-induced inhibition of total proteoglycan synthesis by nonosteoarthritic human articular cartilage chondrocytes in cell culture.[153] Similarly, undefined mixtures of chondroitin sulfates (10 mcg/mL) prevented IL-1β-induced inhibition of total proteoglycan synthesis[158] and IL-1β-induced stimulation of stromelysin-1 activity[153] in cultures of nonosteoarthritic human articular cartilage chondrocytes. Individually, both chondroitin 4-sulfate and chondroitin 6-sulfate significantly inhibited the secretion of the endopeptidase, cathepsin B, by cultured nonosteoarthritic rabbit articular chondrocytes.[159] However, very low concentrations of undefined mixtures of chondroitin sulfates (<1 mcg/mL) failed to significantly inhibit PGE_2 secretion or bacterial LPS-induced production of NO by nonosteoarthritic equine articular cartilage tissue explants in organ culture either alone or when added to significantly inhibitory concentrations of glucosamine-HCl.[137] In contrast, concurrent exposure of chondrocytes harvested from nonosteoarthritic articular cartilage and grown in cell culture to chondroitin sulfate (100 mcg/mL) partially inhibited the pro-apoptotic effect of NO added in amounts that when added alone significantly increased the percentage of cultures chondrocytes undergoing apoptosis.[160] In the same model system, although concurrent exposure was ineffective, the addition of chondroitin sulfate to the culture medium 72 hours before the addition of sodium nitroprusside (SNP) prevented SNP-induced stimulation of NO production and cellular apoptosis.[161]

Diets supplemented with mixed chondroitin sulfates prevented chymopapain-induced degradation of knee articular cartilage in nonosteoarthritic rabbits.[162] Dietary supplementation with chondroitin 6-sulfate (100 mg/kg body weight daily) significantly inhibited the destruction of articular cartilage following subsequent injection of type II collagen in Freund's adjuvant on day 14 in nonosteoarthritic mice[163] and significantly

inhibited the depletion of proteoglycans in articular cartilage following subsequent injection of bradykinin on day 14 in nonosteoarthritic rats.[164]

Fragments of large chondroitin sulfate chains similar to those found in the blood after the oral ingestion of large chondroitin sulfate chains and their degradation prior to the absorption of the fragments significantly inhibited directional chemotaxis, phagocytosis and cytokine-stimulated release of lysozymes in human leukocytes in vitro.[131] Both polydisperse mixtures of chondroitin sulfates[165,166] and Matrix® (a defined mixture of 25% chondroitin 4-sulfate and 75% chondroitin 6-sulfate[167]) inhibited the activity of human leukocyte elastase *in vitro*. The inhibitory activity was limited to chondroitin sulfate polymers larger in size than 2000 daltons,[168,169] may be limited to chondroitin 6-sulfate[170] and increased with the degree of sulfation of the polymers.[169,171] Although only an indirect indicator of events in cartilage, but consistent with an anticatabolic role for supplemental chondroitin sulfates, plasma elastase activity was significantly decreased in nonosteoarthritic rats following 8 days of dietary supplementation with a mixture of chondroitin sulfates (600 mg/kg body weight daily).[5] A report that *in vitro* both chondroitin 4-sulfate and chondroitin 6-sulfate compete with hyaluronan for digestion by hyaluronidase suggests an additional role for supplemental chondroitin sulfates in the direct protection of articular cartilage extracellular matrix macromolecules from the elevated degradative enzyme activities characteristic of asymptomatic subclinical cartilage degeneration.[172]

Supplemental D-Glucosamine and Osteoarthritis

Dietary supplementation with D-glucosamine-HCl (2000 mg/day) produced a significantly greater decrease in subjective pain assessment in 12 weeks than did placebo in adults with regular knee pain that had not yet progressed to clinically identifiable osteoarthritis.[173] However, there were no significant differences in the improvement in clinical or functional tests of joint motion and balance. In contrast, dietary supplementation with D-glucosamine-HCl (1500 mg/day) for 2 months was no better than the consumption of placebo in relieving joint pain in individuals with mild to severe femorotibial osteoarthritis.[174,175]

On the other hand, in 2 randomized double-blind placebo-controlled clinical studies, compared to the effects of placebo, dietary supplementation of subjects with mild to severe femorotibial osteoarthritis with crystalline D-glucosamine sulfate (Dona®; 1500 mg/day) for 1 month has produced significantly greater reductions in articular pain, tenderness, swelling and restriction of movement.[176,177] In another study, short-term dietary supplementation with Dona® (1500 mg/day) 4 weeks produced a significantly greater decrease in the Lequesne functional index of impairment and a significantly greater increase in the percentage of "responders" (subjects experiencing a decrease of at least 3 points in the Lequesne index) than did placebo.[178] Compared to subjects consuming placebo, subjects consuming D-glucosamine sulfate experienced no

differences in the incidence or severity of side effects or in the results of routine clinical chemistry, hematology, urinalysis, heart rate, blood pressure or body weight. Similarly, dietary supplementation with Dona® (1500 mg/day) for 6 to 8 weeks produced significantly greater decreases in joint pain, tenderness and swelling and in the number of days until improvement was noted in joint pain, tenderness or swelling as well as significantly greater increase in the percentage of patients experiencing some degree of improvement in joint pain, tenderness or swelling without producing differences in the incidence or severity of side effects or in hematologic or urinary variables, compared to the effects of placebo consumption.[179]

In long-term randomized double-blind placebo-controlled clinical studies, compared to the effects of placebo, 3 years of dietary supplementation with Dona® (1500 mg/day) by subjects with mild to severe femorotibial osteoarthritis produced significantly greater reductions in the mean rate of femorotibial joint space narrowing (measured as the width of the medial femorotibial joint space, with the knee in full extension, by visual inspection; the "preferred, gold standard outcome" in studies of osteoarthritis[180]), the WOMAC total pain score, the WOMAC indices of total knee health, pain, function and stiffness, the Lequesne functional index and pain assessed by a visual analog scale.[180,181] In addition, the number of subjects experiencing "severe" (i.e., > 0.5 mm) joint space narrowing was significantly smaller after 3 years of dietary supplementation with D-glucosamine sulfate. However, among those subjects consuming D-glucosamine sulfate, those with less severe osteoarthritis at baseline tended to experience better responses. Furthermore, 3 years of daily dietary supplementation with 1500 mg of D-glucosamine sulfate produced no greater number or severity of side effects, including changes in the results of routine annual clinical laboratory examinations, than did 3 years of consumption of placebo.

In a far-ranging multicenter open-label study, a total of 1208 evaluable subjects were supplemented with Dona® (1500 mg/day) for 13 to 99 days.[182] Physician ratings of subject responses were highly favorable: "good" (59% of subjects), "sufficient" (36% of subjects) and "insufficient" (5% of subjects). The best response was experienced by subjects with osteoarthritis of the knee or elbow, while those with osteoarthritis of the hip fared more poorly. The effect of D-glucosamine sulfate on pain scores was directly proportional to the duration of supplementation. In a more targeted open-label study, 69 young athletes (mean age 19 years) with cartilage degeneration of the knee (biochemically similar to osteoarthritis) received dietary supplementation with Dona® (1500 mg/day for 4 days, then 750 mg/day for 90 to 120 additional days.[183] After 120 days, complete remission of symptoms (patella-grinding sound, patella-displacement pain, patella-pressure pain) was reported for 76.5% of the subjects.

In two uncontrolled studies, subjects with femorotibial osteoarthritis were supplemented with either Dona® (1500 mg/day) or ibuprofen (1200 mg/day) for 4 weeks.[184,185] In both studies, both groups experienced similar significant decreases in the Lequesne index of functional impairment[184] and in pain at rest, pain during movement, pain under loading and joint swelling[185] (compared to baseline). However, in both studies there were

significantly more adverse events and adverse event-related trial dropouts among the subjects consuming ibuprofen. In a similar uncontrolled study, subjects with femorotibial osteoarthritis were supplemented with either Dona® (1500 mg/day) or ibuprofen (1200 mg/day) for 8 weeks.[186] In this study, Dona® produced a significantly greater decrease in subjective assessment of knee pain with no difference in the incidence or severity of side effects.

Among subjects with osteoarthritis of the temporomandibular joint, D-glucosamine sulfate (Jamieson™; Windsor, Ontario, Canada; 1500 mg/day for 90 days) supplementation produced a significantly greater decrease in pain assessed using a visual analog scale compared to the pain relief afforded by ibuprofen (1200 mg/day for 90 days).[187] There were no significant differences between D-glucosamine sulfate and ibuprofen in the production of significant reduction in masticatory muscle pain and significant increases in pain-free mouth opening and voluntary mouth opening.

Several groups of investigators have applied the techniques of meta-analysis to evaluate dietary supplementation with D-glucosamine sulfate. One investigator concluded that the randomized double-blind placebo-controlled studies of adequate quality to include in the analysis demonstrated that dietary supplementation with D-glucosamine sulfate produced significantly greater reductions in the Lequesne Index of functional impairment, the severity of pain assessed using a visual analog scale and voluntary consumption of NSAID's for rescue from pain than did placebo (the effect sizes were "large").[188] In addition, it was concluded that D-glucosamine sulfate has demonstrated a consistently excellent safety profile.

Other investigators concluded that dietary supplementation with D-glucosamine sulfate by individuals with osteoarthritis consistently produced significant decreases in joint pain and significant increases in joint function of small-to-moderate magnitude,[189] that dietary supplementation with D-glucosamine sulfate is "probably effective in osteoarthritis in reducing pain and in improving joint function"[107] and that dietary supplementation with D-glucosamine sulfate (1500 mg/day) produces significantly increased pain relief in individuals with femorotibial osteoarthritis accompanied by an excellent safety profile.[190]

When only "high quality" studies were considered by another investigator, it was concluded that dietary supplementation with D-glucosamine sulfate by individuals with osteoarthritis produced an approxmately 50% reduction in pain with a similar improvement in function (a "large" effect consistently greater than that of placebo).[191] However, it was noted that the quality of most published studies concerning dietary supplementation with D-glucosamine sulfate by individuals with osteoarthritis has been generally poor and that the magnitude of the reported effects of dietary supplementation with D-glucosamine sulfate are likely to be inflated by weaknesses in the study designs and analysis. Nonetheless, it was concluded that, despite their poor flaws, the available published studies demonstrate a significant degree of efficacy for dietary supplementation with D-glucosamine sulfate.[191]

Supplemental Chondroitin Sulfate and Osteoarthritis

In randomized, double-blind, placebo-controlled studies of subjects with femorotibial osteoarthritis ranging from mild to severe, compared to the effects of placebo, dietary supplementation with Condrosulf® (Sanova Pharma, Vienna, Austria; 55% chondroitin 4-sulfate, 38% chondroitin 6-sulfate, 5% unsulfated chondroitin sulfates, 1% disulfated chondroitin sulfates, 1% non-chondroitin compounds, average molecular weight: 24,000 daltons; 800 mg/day for 6 months,[192] 1 year[193] or 2 years[194] or 1200 mg/day for 3 months[195] or 6 months[196]) consistently produced significantly greater decreases in the Lequesne Index of functional impairment and in the severity of spontaneous joint pain assessed using a visual analog scale. After 1 year, subjects consuming 800 mg/day also exhibited significantly smaller increases in serum concentrations of osteocalcin (a biomarker of new bone formation) and keratan sulfate (a biomarker pf proteoglycan degradation) and urinary excretions of pyridinoline and deoxypyridinoline (biomarkers of collagen degradation), although overall mobility and the mean rate of narrowing of the internal femorotibial space were not affected by dietary supplementation with chondroitin sulfates.[193] However, subjects consuming 800 mg/day for 2 years exhibited significantly smaller decreases in the mean internal femorotibial space.[194] In none of these studies did dietary supplementation with chondroitin sulfates produce a significant increase in the incidence or severity of side effects.[192-196]

In a randomized, double-blind, placebo-controlled study of subjects with osteoarthritis of the hip ranging from mild to severe, daily supplementation with Condrosulf® (1200 mg for 24 weeks) produced significantly greater decreases in the Lequesne Index of functional impairment, the severity of spontaneous joint pain assessed using a visual analog scale and the voluntary use of nonsteroidal anti-inflammatory drugs (NSAID's) for rescue from pain, although in this short-term study the mean rate of narrowing of the internal femorotibial space was not affected by dietary supplementation with chondroitin sulfates.[197] In a randomized, double-blind, placebo-controlled study of subjects with osteoarthritis of the interphalangeal joints, daily supplementation with Condrosulf® (1200 mg for 3 years) produced a significantly greater decrease in the number of subjects whose osteoarthritis had progressed.[198] However, there was no effect on the progression of osteoarthritis of the metacarpal joints. Even after as long as 3 years of daily supplementation there were no significant differences in the incidence or severity of side effects or adverse reactions.[198]

In three short-term open-label studies, dietary supplementation with Condrosulf® (400 mg/day,[199] 800 mg/day[200] or 1200mg/day[201] for 3 months) significantly reduced the severity of spontaneous joint pain assessed using a visual analog scale and the voluntary use of nonsteroidal anti-inflammatory drugs (NSAID's) for rescue from pain in subjects with osteoarthritis of the interphalangeal, femorotibial or hip joints. One study reported 97% subject compliance with supplementation[200] and no study reported clinically significant side effects.

In a randomized, double-blind, placebo-controlled study of subjects with early mild femorotibial osteoarthritis, 2 years of dietary supplementation with Matrix® (25% chondroitin 4-sulfate and 75% chondroitin 6-sulfate; IRBI S.p.A., Rome, Italy; 800 mg/day) produced significantly greater decreases in the severity of spontaneous joint pain assessed using a visual analog scale and the voluntary use of nonsteroidal anti-inflammatory drugs (NSAID's) for rescue from pain.[202] In another randomized, double-blind, placebo-controlled study of subjects with early mild femorotibial osteoarthritis, 330 days of dietary supplementation with Matrix® (200 mg/day) produced significantly greater decreases in the severity of spontaneous joint pain assessed using a visual analog scale, pain on passive movement, pain on active movement, and pain in the evening; significantly greater increases in joint mobility and ambulation; and a significantly smaller decrease in mean articular cartilage thickness (measured echographically).[203] In neither trial were the incidence or severity of side effects or adverse reactions significantly different among the subjects consuming Matrix® or placebo. Similarly, in an open label pilot study, dietary supplementation with Matrix® (1200 mg/day for 2 months) significantly reduced the severity of spontaneous joint pain assessed using a visual analog scale without producing side effects.[204]

Subjects with femorotibial osteoarthritis ranging from mild to severe have participated in three studiess during which one group of subjects received supplemental Condrosulf®(800 mg/day) for 3 months, placebo for 3 months, Condrosulf®(800 mg/day) for 3 months and placebo for 3 months while a second group of subjects consumed only placebo for 12 months.[205-207] In all 3 studies, after 12 months, despite having consumed only placebo for the 3 months prior, subjects previously supplemented with Condrosulf® exhibited significantly greater decreases in the Lequesne Index of functional impairment, the severity of spontaneous joint pain assessed using a visual analog scale and the mean rate of narrowing of the internal femorotibial space. In one study, the supplemented subjects also exhibited significantly smaller decreases in femorotibial joint articular surface area, femorotibial joint space volume and femoral articular cartilage thickness.[206] In addition, between months 9 and 12 in this study (when both groups consumed placebo), unlike the subjects consuming placebo for 12 months, the subjects previously consuming Condrosulf® did not experience rebound increase in pain.[206] In another study, after 12 months, despite having consumed only placebo for the 3 months prior, subjects previously supplemented with Condrosulf® exhibited significantly smaller increases in the serum concentration of cartilage oligomeric matrix protein (COMP) (a biomarker of synovial and cartilage inflammation), serum concentration of keratan sulfate (a biomarker of proteoglycan degradation), and urinary excretion of pyridinoline and deoxypyridinoline (biomarkers of collagen degradation), confirming the anticatabolic properties of supplemental chondroitin sulfates.[205] In contrast, serum concentrations of osteocalcin were not significantly affected, suggesting that supplemental chondroitin sulfates do not actively influence bone metabolism or turnover.[205] In none of the 3 trials were the incidence or severity of side effects or

adverse reactions significantly different among the subjects consuming Condrosulf® or placebo.[205-207]

The effectiveness of dietary supplementation with Condrosulf® in reducing pain has been compared directly to the effectiveness of NSAID's. Subjects with femorotibial osteoarthritis ranging from mild to severe were supplemented with either Condrosulf®(1200 mg/day) or diclofenac sodium (150 mg/day) for 3 months, after which both groups of subjects were supplemented with placebo for another 3 months.[208] After 3 months of active supplementation, both groups of subjects exhibited similar significant decreases (compared to baseline) in the Lequesne Index of functional impairment, the severity of pain at rest assessed using a visual analog scale and the severity of pain on loading assessed using a visual analog scale. However, after 3 subsequent months of placebo, pain increased significantly in subjects previously supplemented with doclofenac sodium but did not increase in subjects previously supplemented with Condrosulf®.

In two preliminary open-label studies, the diets of subjects with osteoarthritis of the femorotibial or hip joints were supplemented with 1000 mg/day to 1500 mg/day of Structum® (Smith Kline Corp. Philadelphia, PA; undefined polydisperse mixture of chondroitin 4-sulfate and chondroitin 6-sulfate) for up to 4 months.[209,210] Supplementation was reported to have produced significant decreases in pain, with up to 85% of subjects reporting reduced pain and enhanced mobility.[210] When the diets of similar subjects were supplemented with 1000 mg/day of Structum® for 6 months, pain at rest disappeared completely in 57% of subjects with femorotibial osteoarthritis and in 46% of subjects with osteoarthritis of the hip and pain with movement disappeared completely in 17% of subjects with femorotibial osteoarthritis and in 13% of subjects with osteoarthritis of the hip.[211] During the 6 months of this open-label study, joint function improved significantly while voluntary consumption of NSAID's for rescue from pain decreased significantly. In a similar 3-month placebo-controlled study, 1000 mg/day of Structum® produced significantly greater decreases in the Lequesne Index of functional impairment, the severity of pain at rest assessed using a visual analog scale and voluntary consumption of NSAID's for rescue from pain.[212] After 3 subsequent months of discontinuation of supplementation, no significant return of pain occurred among the previously-supplemented subjects. There were no clinically significant side effects in any reported study of dietary supplementation with Structum®.[209-212]

In a randomized placebo-controlled study of subjects with femorotibial osteoarthritis, dietary supplementation with mixed chondroitin sulfates (1000 mg/day; unknown source) for 3 months produced significantly greater decreases in the Lequesne Index of functional impairment and the severity of pain at rest assessed using a visual analog scale.[213] No significant differences occurred in the severity of pain with activity assessed by use of a visual analog scale or in the incidence or severity of side effects. Among the previously-supplemented subjects, pain had not returned three months after discontinuation of supplementation. In a randomized placebo-controlled study of subjects with severe

osteoarthritis of the proximal interphalangeal joints, dietary supplementation with mixed chondroitin sulfates (800 mg/day; unknown source) for 2 years produced significantly greater decreases in the depth of erosions of the femoral articular surface and in the number of painful joints per subject.[214]

Subjects with severe osteoarthritis of the proximal interphalangeal joints received dietary supplementation with Chondral® (a polydisperse mixture of chondroitin sulfates; Societa Prodotti Antibiotici, Milan, Italy; 800 mg/day) either with or without naproxen (500 mg/day).[215] After 2 years, the combination of Chondral® and naproxen produced significantly slower progression of cartilage erosions, but the progression of clinical osteoarthritis was not arrested.

Several groups of investigators have applied the techniques of meta-analysis to evaluate dietary supplementation with chondroitin sulfates. One group concluded that 7 randomized double-blind placebo-controlled studies were of adequate quality to include in their analysis.[216] These studies demonstrated that when consumed at 1200 mg daily for at least 120 days, dietary supplementation with chondroitin sulfates produced significantly greater reductions in the Lequesne Index of functional impairment and in the severity of pain assessed using a visual analog scale than did placebo (the effect size was "large").[216] In addition, 65% of subjects consuming chondroitin sulfates will be expected to benefit more than if they were consuming placebo and, in general, adverse effects were more frequent when placebo was consumed than when chondroitin sulfates were consumed.[216] Other investigators concluded that dietary supplementation with chondroitin sulfates by individuals with osteoarthritis consistently produced significant decreases in joint pain and significant increases in joint function of small-to-moderate magnitude[189] and that dietary supplementation with chondroitin sulfates is "probably effective in osteoarthritis in reducing pain and in improving joint function."[107]

When only "high quality" studies were considered by another investigator, it was concluded that dietary supplementation with chondroitin sulfates by individuals with osteoarthritis produced an approximately 50% reduction in pain with a similar improvement in function (a "large" effect consistently greater than that of placebo).[191] However, it was noted that the quality of most published studies concerning dietary supplementation with chondroitin sulfates by individuals with osteoarthritis has been generally poor and that the magnitude of the reported effects of dietary supplementation with chondroitin sulfates are likely to be inflated by weaknesses in the study designs and analysis. Nonetheless, it was concluded that, despite their flaws, the available published studies demonstrate a significant degree of efficacy for dietary supplementation with chondroitin sulfates.[191]

The application of a pharmacokinetic-pharmacodynamic model of intake-dependent effects resulted in the estimation that dietary supplementation with chondroitin sulfates (especially Condrosulf®, 1200 mg/day) "can reduce baseline pain and algofunctional

indices by over 80%"; it was estimated that about half of this benefit can be experienced in about 35 days of supplementation.[217]

Comparison of Supplemental D-Glucosamine and Chondroitin Sulfate

In an open-label trial, subjects with femorotibial osteoarthritis consumed either D-glucosamine sulfate (unknown source; 1500 mg/day) or mixed chondroitin sulfates (unknown source; 675 mg/day).[218] After 3 months there were no significant differences between the two groups of subjects; 72% of all subjects self-assessed their improvement as "good" without side effects. The extent of improvement in pain during moderate exercise following supplementation was inversely proportional to the severity of pain during moderate exercise prior to the initiation of supplementation (subjects with the most severe pain responded the least to either dietary supplement).

Combination of Chondroitin Sulfate and D-Glucosamine in an Animal Model of Osteoarthritis

In a rabbit surgical model of osteoarthritis, following surgery five groups of rabbits were fed either a control diet or the control diet supplemented with either D-glucosamine HCl, mixed chondroitin sulfates, manganese ascorbate or Cosamin® for 16 weeks.[219] Upon sacrifice, the rabbits fed either the control diet or the control diet supplemented with either D-glucosamine HCl, mixed chondroitin sulfates, or manganese ascorbate exhibited no significant differences in the area of articular cartilage surface lesions, the percentage of animals exhibiting severe lesions, the rates of synthesis or degradation of articular cartilage proteoglycans or the magnitude of IL-1β-induced collagenase activity. In contrast, dietary supplementation with Cosamin® produced significantly greater decreases in the area of articular cartilage surface lesions, the percentage of animals exhibiting severe lesions and the magnitude of IL-1β-induced collagenase activity. In this model, Cosamin® had no effect on the rates of synthesis or degradation of articular cartilage proteoglycans.

Combinations of Chondroitin Sulfate and D-Glucosamine and Osteoarthritis

In a randomized double-blind placebo-controlled clinical trial, subjects with mild to moderate femorotibial osetoarthritis supplemented their diets with either a combination of D-glucosamine-HCl (1000 mg/day), mixed chondroitin sulfates (800 mg/day) and manganese ascorbate (152 mg/day) (2 tablets of Cosamin®; Nutramax Laboratories, Inc.) or placebo.[220] After 6 months, dietary supplementation with Cosamin® was associated with significantly greater decreases in the Lequesne index of functional impairment, although there was no difference in WOMAC pain scores or in subject self-assessment between subjects supplemented with Cosamin® and those supplemented with placebo. In addition, subjects with severe femorotibial osetoarthritis experienced little improvement.

There also was no difference in the incidence or severity of side effects. In another randomized double-blind placebo-controlled clinical trial, subjects with mild to moderate femorotibial osetoarthritis or osteoarthritis of the lower back supplemented their diets with either a combination of D-glucosamine-HCl (1500 mg/day), mixed chondroitin sulfates (1200 mg/day) and manganese ascorbate (228 mg/day) (3 tablets of Cosamin®; Nutramax Laboratories, Inc.) or placebo.[221] After 16 weeks, Cosamin®-supplemented subjects with femorotibial osetoarthritis exhibited significantly greater decreases in summary disease score, subject self-assessment of pain, severity of pain assessed using a visual analog scale, but there was no difference in maximum running times. Subjects with osteoarthritis of the lower back exhibited no response to the dietary supplement. There were no intergroup differences in the incidence or severity of side effects.

In a randomized double-blind placebo-controlled trial, dietary supplementation with D-glucosamine-HCl (1500 mg/day) plus mixed chondroitin sulfates (1200 mg/day) was compared with placebo in subjects with painful osteoarthritis of the temporomandibular joint.[222] After 12 weeks, subjects supplemented with D-glucosamine-HCl plus mixed chondroitin sulfates exhibited significantly greater decreases in temporomandibular joint tenderness and sounds and in voluntary consumption of pain relieving medications without the production of side effects. In an uncontrolled study of subjects with chronic temporomandibular joint osteoarthritis, 80% of subjects reported self-perceived decreases in temporomandibular joint sounds during dietary supplementation with a combination of D-glucosamine-HCl (1600 mg/day), mixed chondroitin 4- and 6-sulfates (1200 mg/day) and calcium ascorbate (1000 mg/day) (NOW Foods, Glendale Heights, IL).[223]

Daily Intake of Supplemental Glucosamine that is Effective in Reducing the Risk of Osteoarthritis

The reliable and credible scientific literature indicates that daily dietary supplementation with 1500 mg of D-glucosamine sulfate is effective in reducing the risk of osteoarthritis.

When combined with dietary supplementation with chondroitin sulfates (at least 800 mg/day), daily dietary supplementation with 1000 mg of D-glucosamine-HCl is effective in reducing the risk of osteoarthritis.

Daily Intake of Supplemental Chondroitin Sulfate that is Effective in Reducing the Risk of Osteoarthritis

The reliable and credible scientific literature indicates that daily dietary supplementation with 1200 mg of chondroitin sulfate (containing chondroitin 4-sulfate and chondroitin 6-sulfate in approximately equal proportions), whether consumed alone or in combination with supplemental D-glucosamine sulfate or D-glucosamine-HCl is effective in reducing the risk of osteoarthritis.

Safety of Daily Intakes of Supplemental Chondroitin Sulfate and D-Glucosamine that are Effective in Reducing the Risk of Osteoarthritis

D-Glucosamine is present in all foods containing cartilage or glycoproteins.[17] It has not been possible to estimate an LD_{50} for oral D-glucosamine sulfate because no deaths have occurred in mice and rats from intakes of up to 5000 mg/kg body weight.[224] Daily dietary supplementation with 2149 mg of D-glucosamine sulfate per kg body weight produced no systemic or gastrointestinal adverse reactions in dogs.[141] Horses fed 8 g of D-glucosamine-HCl daily for 48 weeks (equivalent to about 16 mg/kg body weight daily in an adult human) exhibited no effects on bone metabolism.[225] In humans, intra-articular (200 mg)[226] or intramuscular injection of D-glucosamine sulfate (200 mg once[226] or 400 mg daily for 7 days[227,228]) produced no adverse reactions. Dietary supplementation with D-glucosamine sulfate[176-181] or D-glucosamine-HCl[173-175,220-222] for up to 3 years did not produce an increase in the incidence or severity of side effects in placebo-controlled human studies.

The most common side effects reported by humans consuming D-glucosamine sulfate include reversible epigastric pain, epigastric tenderness, heartburn, nausea, diarrhea, dyspepsia, vomiting, constipation, drowsiness, headaches, and mild skin reactions.[182,185] Oral D-glucosamine sulfate does not interfere with the efficacy of medications for cardiovascular, liver, or lung diseases, diabetes or depression[227] or produce insulin resistance in rats[229] or humans.[181,230] However, oral D-glucosamine sulfate may potentiate active peptic ulcers.[182] Obesity may reduce responsiveness to dietary supplementation with D-glucosamine sulfate.[182]

In rats and rabbits, oral chondroitin sulfate polymers (1 g/kg body weight daily) produced no effects on mutagenesis or reproductive function.[5] In the isolated rabbit intestinal loop model of the digestive tract, no change in the amplitude of intestinal contractions or in the tonicity of the intestine was noted in the presence of 1 to 3 mg/mL chondroitin sulfate polymers. At intakes of 0.25 to 1.0 g/kg body weight, no change occurred in the rate of intestinal transit in mice. Chondroitin sulfate polymers do not modify the coagulation time. Intravenous chondroitin sulfate polymers (25 to 100 mg/kg body weight, perfused at a rate of 25 mg/min) had no effect on the human electrocardiogram; 100 mg/kg induced a slight and transitory decrease of arterial pressure. However, chondroitin sulfate polymers can cause an increased respiratory rate and amplitude. No change in the volume or the electrolyte concentration of human urine was found after subcutaneous administration of 100 mg/mL of chondroitin sulfate polymers.[5]

In an analysis of 16 human studies that included a total of 372 subjects, it was concluded that dietary supplementation with chondroitin sulfate polymers (800 mg/day) did not produce more adverse events than did placebo.[231] Dietary supplementation with chondroitin sulfate polymers for up to 2 years did not increase the incidence or severity of side effects in placebo-controlled human studies.[192-198,202,203,205-207,212-214,220-222]

Conclusions

- Maintaining the structural and functional integrity of the proteoglycan component of the extracellular matrix of articular cartilage is required for preservation of healthy joint architecture and biomechanics.

- Imbalanced metabolism favoring catabolism within the extracellular matrix of articular cartilage produces degenerative changes in the proteoglycan composition of the matrix.

- Compromise of the structural and functional integrity of the proteoglycan component of the extracellular matrix of articular cartilage results in net loss of articular cartilage tissue, inferior biomechanical competence and structural deformation of joint architecture.

- Net degradation of the extracellular matrix of articular cartilage, accompanied by the production of spontaneous repair matrix with abnormal proteoglycan composition, results in asymptomatic subclinical osteoarthritic change.

- The progression of asymptomatic osteoarthritic change to osteoarthritis is not inevitable.

- The progression of osteoarthritic change is required in order for abnormalities in articular cartilage composition and structure to progress to osteoarthritis.

- Osteoarthritic change in the absence of joint pain represents a modifiable risk factor for later development of osteoarthritis.

- Dietary supplementation with D-glucosamine, glucosamine-HCl, glucosamine sulfate or chondroitin sulfate contributes to the preservation of articular cartilage, inhibits the initiation of osteoarthritic change in articular cartilage and inhibits the progression of osteoarthritic change to symptomatic osteoarthritis.

- Dietary supplementation with D-glucosamine, glucosamine-HCl, glucosamine sulfate or chondroitin sulfate is an effective modifier of osteoarthritic change and reduces the risk for osteoarthritis.

Summary Conclusions

In conclusion, I find that there is significant scientific agreement in support of the following health claims:

- Glucosamine may reduce the risk of osteoarthritis.
- Chondroitin sulfate may reduce the risk of osteoarthritis.
- Glucosamine and chondroitin sulfate may reduce the risk of osteoarthritis.
- Glucosamine may reduce the risk of osteoarthritis-related joint pain, tenderness and swelling.
- Chondroitin sulfate may reduce the risk of osteoarthritis-related joint pain, tenderness and swelling.
- Glucosamine and chondroitin sulfate may reduce the risk of osteoarthritis-related joint pain, tenderness and swelling.
- Glucosamine may reduce the risk of joint degeneration.
- Chondroitin sulfate may reduce the risk of joint degeneration.
- Glucosamine and chondroitin sulfate may reduce the risk of joint degeneration,
- Glucosamine may reduce the risk of cartilage deterioration.
- Chondroitin sulfate may reduce the risk of cartilage deterioration.
- Glucosamine and chondroitin sulfate may reduce the risk of cartilage deterioration.

Michael J. Glade, Ph.D., F.A.C.N., C.N.S.
(a copy of my CV is attached)

9/23/03

HEALTH CLAIM PETITION:

DIETARY SUPPLEMENTATION OF CRYSTALLINE GLUCOSAMINE SULFATE (GLUCOSAMINE SULFATE SODIUM CHLORIDE-USP/NF 2003) REDUCES THE RISK OF OSTEOARTHRITS JOINT STRUCTURE DETERIORATION AND RELATED JOINT PAIN AND LIMITATION OF FUNCTION

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September 17, 2003

PETITIONER: Rotta Pharmaceuticals, Inc. (Rotta Research/Rottapharm Group)

ADDRESS: c/o Martin J. Hahn, Esq.
Hogan & Hartson, L.L.P.
555 13th Street NW
Washington, DC 20015

SUBJECT: Health Claim Petition: Dietary supplementation of Crystalline Glucosamine Sulfate (Glucosamine Sulfate Sodium Chloride-USP/NF 2003) reduces the risk of osteoarthritis joint deterioration and related joint pain and limitation of function.

I. INTRODUCTION

We submit this Petition on behalf of our client, Rotta Pharmaceuticals Inc. (hereinafter "Petitioner") pursuant to section 403 (r) (5) (D) of the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. § 343 (r) (5) (D)) and Food and Drug Administration (FDA) procedures for the review of "qualified health claims" described in Agency guidance for Industry and FDA (68 Fed. Reg. 41387 (July 11, 2003)). The Petitioner respectfully requests that FDA approve for use in the labeling of Crystalline Glucosamine Sulfate (Glucosamine Sulfate Sodium Chloride-USP/NF 2003) a health claim communicating that this substance can reduce the risk of osteoarthritis joint structure deterioration and related joint pain and limitation of function. All of the items specified in 21 C.F.R. § 101.70 (f) are included or attached to this Petition.

The proposed health claim responds to a major public health concern in the United States, osteoarthritis, the most common form of arthritis (1). Osteoarthritis is a serious and degenerative joint disease that generally is characterized by clinical evidence of both joint structural changes and joint pain (1). In the United States, symptomatic osteoarthritis in the knee is estimated to affect six percent of adults aged

30 years and over and symptomatic hip osteoarthritis is estimated to affect roughly three percent (2). Because the prevalence of osteoarthritis increases with age, it is expected that the disease will become even more prevalent as the population ages.

As will be explained more fully below, the Petitioner believes that the proposed health claim is supported by significant scientific agreement within the meaning of section 403(r)(3)(B) of the FFDCA. To the extent that the agency disagrees that there is significant scientific agreement in support of the proposed health claim, the Petitioner would be willing to have the agency review the proposed claim as a "qualified health claim," as described in agency guidance of July 11, 2003. In light of the extensive research to date addressing the relationship between crystalline glucosamine sulfate and osteoarthritis, the proposed claims are, at a minimum, "Category B" claims for which the scientific evidence may be described as supportive but not conclusive.

Rotta Pharmaceuticals Inc. is a fully owned subsidiary of the multinational Rotta Research/Rottapharm Group, which developed crystalline glucosamine sulfate for use in osteoarthritis and sponsored many of the clinical trials summarized in this Petition. An overwhelming majority of the published clinical trials evaluating the effect of glucosamine on osteoarthritis have been performed using Rotta Research/Rottapharm Group's source of crystalline glucosamine sulfate. These clinical trials represent the largest body of evidence supporting the use of glucosamine in the prevention of osteoarthritis.

There are limited clinical studies evaluating the effect of other sources of glucosamine on the prevention of osteoarthritis. Other sources of glucosamine do not

share the same quality, pharmacological, and pharmacokinetic properties of crystalline glucosamine sulfate. More importantly, these other sources of glucosamine have not been shown through clinical trials to have the same effect on osteoarthritis as crystalline glucosamine sulfate. Indeed, the scientific literature contains several statements cautioning against the generalization of data on crystalline glucosamine sulfate to support the efficacy of other sources of glucosamine. Simply stated, there are insufficient data to support the inclusion of other sources of glucosamine in the proposed health claim.

Based on our understanding of the agency guidelines on qualified health claims, we believe that there may be sufficient data to support the classification of other sources of crystalline glucosamine sulfate for a "category D" or possibly a "category C" qualified health claim. Because this Petition focuses on crystalline glucosamine sulfate, we do not provide an analysis of whether there are sufficient data to support a qualified health claim for other sources of glucosamine, and if so, whether there are sufficient data to justify placement in "category D" or perhaps "category C."

II. PRELIMINARY REQUIREMENTS

The proposed crystalline glucosamine sulfate/osteoarthritis health claim satisfies all applicable "Preliminary Requirements" under FDA rules, as set forth in 21 C.F.R. §§ 101.70(f) and 101.14(a)-(b). These regulations require that the subject of a health claim be a "substance," as defined in 21 C.F.R. § 101.14(a), the substance be associated with a disease or health-related condition of public health significance, the substance contribute taste, aroma, nutritive value, or certain other technical or functional effects specified by FDA, and the substance be demonstrated to be safe and lawful under the applicable food safety provisions of the Federal Food, Drug, and Cosmetic Act (FFDCA).

A. Crystalline Glucosamine Sulfate Qualifies as a "Substance"

Crystalline glucosamine sulfate is marketed as a dietary supplement and is subject to regulation as a "food" in accordance with section 201(f) of the FFDCA. "Substance" is defined in the FDA regulations as "a specific food or component of food, regardless of whether the food is in conventional food form or a dietary supplement that includes vitamins, minerals, herbs, or other similar nutritional substances" (21 C.F.R. § 101.14(a)). As a lawfully marketed dietary supplement, crystalline glucosamine sulfate falls within the definition of "substance."

B. Crystalline Glucosamine Sulfate is Associated with Osteoarthritis

Crystalline glucosamine sulfate is associated with osteoarthritis, a disease for which the general U.S. population and the elderly (an identified U.S. population subgroup) are at risk. Osteoarthritis is a degenerative joint disease that can potentially

affect all synovial joints (*i.e.*, joints containing a lubricating fluid that is secreted by membranes surrounding the joint). Osteoarthritis involves the degeneration of the articular cartilage, although there may be other contributing factors originating in different joint tissues such as the subchondral bone and the synovium. Surprisingly, the pathogenetic process (biomechanical, biochemical, or other) of this disease is still relatively unknown.

In healthy individuals, articular cartilage is maintained through a dynamic process that balances synthesis and degradation of the cartilage matrix. Degeneration of the articular cartilage is due to an imbalance in this system, which results in a generalized loss of cartilage. Remodelling of the subchondral bone also occurs, as well as mild inflammatory reactive changes in the synovial membrane. These pathological changes of the joint structure can be detected by imaging techniques such as plain radiography, where cartilage loss is usually seen as a decrease in joint space width (joint space narrowing) and by remodelling of the subchondral bone which is evidenced by the presence of osteophytes (bone spurs) and other signs. The degenerative process and the mild synovial inflammation cause joint pain, particularly when the joint is in use, and limit joint function. The scientific evidence in this Petition convincingly establishes that crystalline glucosamine sulfate, when given to individuals diagnosed with osteoarthritis, can prevent further joint degradation, can reverse the symptoms by minimizing the inflammation and restoring articular cartilage, can reduce joint pain and can result in increased joint function. Given the physiological mechanism of action of crystalline glucosamine sulfate and other factors, there also are sufficient data demonstrating the ability of crystalline glucosamine sulfate to be effective in reducing the risk of developing osteoarthritis.

Osteoarthritis is the most common form of arthritis and the most common reason for total hip and total knee replacement (1). Symptomatic disease in the knee is reported to occur in approximately six percent of U.S. adults 30 years of age or older; symptomatic hip osteoarthritis occurs in roughly three percent (2). Because the prevalence of osteoarthritis increases with age, the disease will become even more prevalent as the population ages. Indeed, results of community-based surveys have shown that the general incidence and prevalence of osteoarthritis increase 2- to 10- fold from age 30 to 65 years, with further increases thereafter (3).

Osteoarthritis of the knee is particularly common, with radiographic osteoarthritic changes of the tibiofemoral compartment reported to occur in five percent to 15 percent of people aged 35 to 74 years in the Western world (4). The impact on disability attributable to knee osteoarthritis is similar to that attributed to cardiovascular disease and greater than that caused by any other medical condition in the elderly (5).

C. Crystalline Glucosamine Sulfate Contributes Nutritive Values When Consumed at Levels that are Necessary to Justify the Claim

Crystalline glucosamine sulfate contributes "nutritive value" when consumed at levels necessary to justify the proposed claim and meets the requirement of 21 C.F.R. § 101.14 (b)(3)(i) for substances intended for consumption at other than decreased dietary levels. For purposes of health claims, FDA has defined "nutritive value" to mean "a value in sustaining human existence by such processes as promoting growth, replacing loss of essential nutrients, or providing energy" (21 C.F.R. § 101.14(a)(3)). Crystalline glucosamine sulfate provides nutritive value through its role in the synthesis

of new cartilage and maintenance (through inhibition of catabolic enzymes) of existing cartilage. These functions represent the basis for the clinical effects of crystalline glucosamine sulfate.

Cartilage consists of an extracellular matrix that contains proteoglycans, collagen, and water (6). The proteoglycans are a protein core that contains glycosaminoglycan side chains of varying lengths. Glucosamine is an aminomonosaccharide and is one of the building blocks for the glycosaminoglycans, which can be found in the articular cartilage matrix and synovial fluid. Following oral administration, glucosamine from crystalline glucosamine sulfate is bioavailable to the joint tissues (7) and is preferentially incorporated by the chondrocytes into the components of the glycosaminoglycan chains in the intact cartilage (8). Crystalline glucosamine sulfate stimulates the synthesis of proteoglycans and decreases the activity of catabolic enzymes (9, 10, 11). These activities have been recently related to glucosamine-induced reversal of the negative effects of interleukin-1-stimulated expression of enzymes involved in joint tissue destruction and inflammation, such as metalloproteases, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) (12, 13). This cytokine antagonism is achieved through an inhibitory effect of the interleukin-1 intracellular signalling cascade in chondrocytes, and in particular by the suppression of NF-KB activation (13).

The inorganic sulfates found in crystalline glucosamine sulfate are also believed to contribute to the physiological effects. Sulfates control the rate of synthesis of the glycosaminoglycans and proteoglycans that become a part of the cartilage matrix. The sulfate serum levels increase after glucosamine sulfate administration (14). The

importance of sulfate in the cartilage synthesis process provides further support for using a source of glucosamine that provides sulfates.

D. Crystalline Glucosamine Sulfate Use at the Levels Necessary to Justify the Claim is Safe and Lawful

Crystalline glucosamine sulfate is safe and lawful under the applicable food safety provisions of the FFDCA, as required by 21 C.F.R. § 101.14(b)(3)(ii). The FFDCA requires that dietary ingredients and dietary supplements not present a significant or unreasonable risk of injury under the conditions of use recommended or suggested in the product labelling, or if none, under the ordinary conditions of use (FFDCA § 402(f)). As discussed more fully in Section III.D below, the safety of glucosamine is evidenced by clinical trial data, by its physical properties, chemical structure, and metabolic fate, and through experience based on widespread use throughout the world.

III. SUMMARY OF SCIENTIFIC INFORMATION

This section of the Petition summarizes the publicly available randomized controlled clinical trials examining the use of all formulations of glucosamine in the prevention of osteoarthritis-related joint deterioration, joint pain, and limitation of function. We have divided these clinical studies into four separate categories: (1) systematic reviews and meta-analyses, (2) clinical studies conducted prior to 1994, which recognizably are not of the same quality as those published later, (3) clinical studies conducted after 1994, and (4) studies performed with glucosamine formulations other than crystalline glucosamine sulfate.

A. Systematic reviews and meta-analyses

Since 2000, there have been three publications involving major systematic reviews and meta-analyses examining the available literature on glucosamine (all sources) and osteoarthritis. We summarize the systematic reviews in the order of their publication. Please recognize that the first two reviews were conducted prior to the publication of long-term studies in the *Lancet* (2001) (15) and the *Archives of Internal Medicine* (2002) (16).

1. McAlindon T et al. JAMA 2000; 283 :1469-75 (Reference n. 17)

McAlindon et al. combined a meta-analysis with a systematic quality assessment to evaluate the benefit of glucosamine and, separately, chondroitin preparations in relation to osteoarthritis. The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) funded the analysis. The authors limited their search to placebo-controlled trials of at least four weeks duration that had been published prior to June 1999. The authors identified six glucosamine trials for their review (two of which

did not involve oral administration of glucosamine). Of the six trials reviewed, five involved crystalline glucosamine sulfate. The authors noted moderate treatment effect sizes for glucosamine on osteoarthritis symptoms and recognized that effect sizes were larger when treatment exceeded four weeks.

Although the authors concluded that the effects of glucosamine may be exaggerated, due to quality issues and likely publication bias, these findings must be interpreted with caution. The authors did indeed identify the presence of general quality issues for the trials reviewed, but the average quality scores they calculated for the glucosamine trials were similar to the standards reported for peer-reviewed medical journal articles and, admittedly, to that of other agents used in osteoarthritis. The authors also presented asymmetrical funnel plots (*i.e.*, plots of the trials' effect estimates against sample size) for the trials included in the analysis, which they concluded to be suggestive of publication bias, as explained by Egger et al. (18). The authors failed to note the Egger's group finding (18, 19), however, that funnel plot asymmetry is common (*i.e.*, 38 percent), in meta-analyses published in leading medical journals. (As will be discussed below, Cochrane Reviews tend to be more accurate.)

The Egger's group also acknowledged that asymmetry may frequently be due to factors other than bias, such as true heterogeneity (*i.e.*, true difference of effects between trials). True heterogeneity is very probable in osteoarthritis trials due to differences in patient selection, severity stages, and evaluation methods. Furthermore, the authors depicted a combined funnel plot analysis for glucosamine and chondroitin, but asymmetry seems to be much less pronounced for glucosamine. Finally, the authors admitted they were unable to find evidence of unpublished, negative studies, which

would support a finding of likely bias. The Petitioner also is unaware of any such unpublished studies for crystalline glucosamine sulfate.

Notwithstanding the above observations, the authors concluded that glucosamine indeed exhibited moderate to large effects on osteoarthritis symptoms and they noted that glucosamine had a good safety record.

2. Towheed et al. Cochrane Library, Update Software, Oxford, England; 2001: issue 2 (Reference n. 20)

Towheed et al. published a more accurate and detailed meta-analysis as a Cochrane Review in early 2001. Towheed covered a similar period as McAlindon (1966 to November 1999), but considered a total of 16 randomized controlled trials, including all placebo-controlled trials examined by McAlindon (17). In addition, the Towheed analysis included 4 clinical trials controlled with a reference medication. The inclusion of trials controlled with a reference medication is of particular importance because the standard pharmacological treatment for osteoarthritis symptoms consists of analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs). The trials in this review used NSAIDs as a reference treatment in the control group.

All trials were randomized and double-blinded, and included a large overall population of 2029 patients (992 assigned to glucosamine and 1037 to comparators). The trials were short-term studies for osteoarthritis symptoms, with a mean trial duration of 6.25 weeks. The oral route was used in the vast majority of trials, at a dose equivalent to 1500 mg/day glucosamine sulfate in most instances. Most trials examined the effects of treatment on the knee.

The authors' quality assessment of these studies, using validated methods, resulted in significantly different comments than those reported previously by McAlindon. Indeed, the authors of this review found that collectively, the glucosamine trials were as good, if not better, than NSAID trials in treating osteoarthritis. The authors assigned the glucosamine trials with a total median quality score of 9 (out of a possible 16). Importantly, there was a strong trend for improvement in quality for more recent trials, with those published in the 1990s having a median scored of 12 while those published in the 1980s had a median score of 7.5.

The authors used Standardized Mean Differences (SMDs) as a measure of effect size and in accordance with the literature, interpreted an effect size of 0.20 as small, 0.50 as moderate, and ≥0.80 as large. The pooled SMD for pain reduction vs. placebo was very large at 1.40. The pooled SMD for function, as measured by the Lequesne index, was moderate at 0.63. The moderate rating corresponded to a difference in the change from baseline vs. placebo of 3.5 points (*i.e.*, a change of over one severity class in the Lequesne's handicap scale (21)).

In the four trials comparing crystalline glucosamine sulfate to NSAIDs (there were no such trials involving other sources of glucosamine), the authors reported that glucosamine was equivalent in two and superior in the other two. The pooled SMD (to measure the effect size vs. NSAIDs) for pain reduction was considered large at 0.86 (3 trials) and small for purposes of joint function as measured by the Lequesne index at 0.32 for the 2 trials. The authors noted therefore a trend for superiority when compared to NSAIDs and suggested that the use of glucosamine represented a possible major breakthrough.

The authors concluded that there was good evidence that glucosamine is both effective and safe in treating osteoarthritis. The authors noted that all but one of the trials used crystalline glucosamine sulfate and that the one trial with glucosamine hydrochloride gave less favourable, or at least more variable results. The authors conclude that the glucosamine formulation and the presence of sulfates, therefore, seem to be important. The safety profile of glucosamine was considered excellent by the authors of this Cochrane Review, although the authors remarked that, at that time, clinical trials to test long-term efficacy and safety were needed. (Such studies have since been performed and published.)

3. <u>Richy F. et al. Arch. Inter. Med. 2003 ; 163 : 1541-22 (Reference n. 22)</u>

In the third and most recent meta-analysis, Richy et al. sought to separately evaluate the efficacy and safety of glucosamine sulfate and chondroitin sulfate. The authors restricted their analysis to randomized, double-blind, placebo-controlled trials (thus excluding reference-controlled trials) examining the benefit of oral glucosamine sulfate in knee osteoarthritis, with results expressed by today's state-of-the-art outcomes. These restrictions resulted in only 7 trials with glucosamine sulfate that could be included, only 3 of which had been included in the previous JAMA and Cochrane reviews. Five of the trials involved the Petitioner's crystalline glucosamine sulfate and the remaining two trials involved different formulations of glucosamine sulfate (23, 24).

The Richy meta-analysis has particular merit because it addresses major recommendations noted in the two previous systematic reviews, namely, the need for

(1) further high-quality, large trials and (2) long-term clinical trials. These recommendations have been indeed fulfilled by two independent, high-quality, long-term trials performed with crystalline glucosamine sulfate—one published in the *Lancet* (15) in 2001 and the other in the *Archives of Internal Medicine* in 2002 (16)—that were not available for consideration in the JAMA and Cochrane reviews but were included in this review.

Data were analysed from 1020 patients in glucosamine sulfate trials. The mean quality score of these trials was high: 90 percent according to the scoring method adopted (and significantly higher than in chondroitin trials). The two long-term, three-year, trials of crystalline glucosamine sulfate (15,16) provided consistent results of highly significant evidence of a structural efficacy of crystalline glucosamine sulfate, as assessed by radiographic joint space narrowing. The low to medium effect size on this parameter translated into natural units that expressed a major effect of clinical significance, as explained in detail in the two single study reports (15,16). There were insufficient data to evaluate a possible structural effect of chondroitin.

The global estimators used in this meta-analysis show substantial beneficial effects of crystalline glucosamine sulfate on symptoms of osteoarthritis, whether used in short-term or long-term clinical trials. The corresponding effect sizes tended to be lower than those calculated in the JAMA and Cochrane reviews, because of the more restrictive trial inclusions and the more conservative analysis model, according to the authors. The two studies using other formulations of glucosamine sulfate (23,24) emerged as those with the lowest effect size. Unlike McAlindon's 2000 JAMA meta-

analysis, the authors excluded any effect from possible publication bias on the robustness of the results.

B. Individual clinical trials

Unless otherwise indicated, all individual trials reported in this Petition were randomized, controlled intervention trials. All such trials can be classified or rated as Study Design Type One according to the recent (July 2003) FDA guidance entitled "Interim Evidence-based Ranking System for Scientific Data".

As indicated in the Cochrane Review (20), there is an obvious difference in the quality of the studies performed in the 1980s with those published after 1994, with the later studies being of significantly greater quality. According to the recent FDA guidance document, the earlier studies could be assigned the "?" designator because some uncertainties exist as to whether the studies adequately addressed issues of scientific quality such as inclusion/exclusion, bias, generalizability, and data collection and analysis. The trials published after 1994, however, have adequately addressed these issues and could be assigned the "+" designator.

Our discussion below first summarizes the early clinical trials and then provides a summary of the higher quality studies published after 1994. There is a separate section that summarizes the clinical studies performed on glucosamine formulations other than crystalline glucosamine sulfate. Copies of these clinical studies can be found in Attachment 1, which contains copies of all of the references cited in this Petition.

1. Early clinical trials

a. Placebo-controlled studies

There were four randomized, placebo-controlled, double-blind, parallel-group studies published between 1980 and 1981 (25, 26, 27, 28) that were included in the Cochrane Review (20). Each of these four studies used crystalline glucosamine sulfate at an oral dose equivalent to 1500 mg/day of glucosamine sulfate. At the time of these studies, the pharmacokinetic studies (7) had not yet demonstrated the ability to administer crystalline glucosamine sulfate once per day. These studies involved the administration of a 500 mg dose three times per day.

Two of the studies (25, 26) evaluated oral administration only. Drovanti et al. (25) treated two parallel groups of 40 hospitalized patients (80 total patients) with osteoarthritis at different joint localizations for 4 weeks. Pujalte et al. (26) treated for six to eight weeks two groups with 12 out-patients in each group (24 total patients) that had been diagnosed with osteoarthritis of the knee. The studies used the Likert scale to assess the effects of treatment. Both studies reported a statistically significant decrease in Likert scales scores of the crystalline glucosamine sulfate treated group when compared to the placebo group in joint pain, tenderness, swelling, and movement limitation. In addition, 70-80 percent of the patients in the treated groups showed positive results compared to only 20-40 percent positive results in the placebo groups. Significant clinical improvement was reached in the active group after the second week of treatment. Tolerability was good in both studies with no differences between the treatment and placebo groups. Drovanti (25) also reported no detected abnormalities in fecal occult blood analysis, indicating good tolerance of the gastrointestinal tract for crystalline glucosamine sulfate.

The other two clinical studies (27,28) had identical designs and outcomes and further support the findings of the previous studies. Crolle and D'Este (27) and D'Ambrosio et al. (28) treated two small groups (30 overall in each study) of in-patients with either parenteral crystalline glucosamine sulfate (400 mg/day glucosamine sulfate) or a reference medication for a one-week period that was followed by a two-week treatment with an oral dose of either placebo or 1500 mg/day of glucosamine sulfate. The reports provide little information on the localization of the osteoarthritis in these patients. The studies report that the initial improvement on the usual pain and functional parameters during the parenteral administration was maintained and even increased during the oral treatment with crystalline glucosamine sulfate. These findings further support the efficacy of the glucosamine sulfate 1500 mg/day oral dose. The studies also reported good tolerability for crystalline glucosamine sulfate, which is particularly relevant because the patients in the Crolle and D'Este (27) study had serious concomitant diseases and consequent treatments that were not affected by the crystalline glucosamine sulfate therapy.

b. <u>NSAID-controlled studies</u>

Vaz (29) conducted the first controlled study comparing glucosamine with an NSAID. This randomized, double-blind study had two parallel groups of 20 knee osteoarthritis patients (40 total patients). In this eight-week study, one group received an oral dose of 1500 mg/day of glucosamine sulfate and the other group received 1200 mg/day of ibuprofen. Pain relief tended to be slightly better in the crystalline glucosamine sulfate group than in the group receiving oral ibuprofen. The kinetics of the effect, however, differed. The NSAID group reached its maximum activity within the first two weeks and then remained stable. The crystalline glucosamine sulfate group

attained the same level of efficacy as the ibuprofen group after the third week and showed a trend for a continuous linear improvement. Tolerability also tended to be better for crystalline glucosamine sulfate, although it did not reach statistical significance. Vaz reported no differences at fecal occult blood testing, which was normal in most patients.

c. <u>Other early clinical studies</u>

Although not fully relevant to this Petition because it involved the intraarticular route of administration of crystalline glucosamine sulfate, the study by Vajaradul (30) is included in both the JAMA and Cochrane meta-analysis and is therefore briefly reviewed here. A total of 54 patients with knee osteoarthritis (out of 60) completed a 5-week treatment course with weekly intraarticular injections of either glucosamine or placebo. Knee pain decreased to a significantly greater extent and joint function improved in the treatment group. The authors also report good systemic and local safety.

The Cochrane Review also mentions publication of a post-marketing surveillance study by Tapadinhas et al. (31). This study involved 1208 patients with osteoarthritis at different joint localizations that were treated with crystalline glucosamine sulfate in an open fashion by 252 physicians throughout Portugal. The treatment lasted for six to eight weeks with a mean of 50 days and a range from 13 to 99 days. This study is not a randomized, controlled intervention trial. The authors report that a treatment response was obtained in over 90 percent of patients and that, consistent with other published studies, the effects of crystalline glucosamine sulfate persisted after cessation of treatment. The authors also reported good results on the safety of crystalline glucosamine sulfate, which is significant because the study involved patients receiving

previous and concomitant treatments for other diseases. Only 12 percent of patients experienced adverse effects with reactions that were generally mild, reversible, and predominantly affected the gastrointestinal tract.

2. **Recent pivotal clinical trials**

a. **Short-term pivotal trials vs. placebo or vs. NSAIDs**

i. Noack W et al. Osteoarthritis Cart 1994; 2: 51-59 (IV.B.10 – Reference n. 32)

In a large randomized, placebo-controlled, double-blind, parallel-group study, Noack et al. evaluated the symptom efficacy and safety of crystalline glucosamine sulfate (equivalent to 1500 mg/day glucosamine sulfate). This study involved two groups of 126 patients (252 total patients) with knee osteoarthritis that received either crystalline glucosamine sulfate or placebo over a 4-week treatment period. The baseline Lequesne index for the patients at the start of the study was between 10 and 11 points, indicating that the symptoms were moderate to severe.

There was a decrease in the Lequesne index by over 3 points after 4 weeks in the patients receiving crystalline glucosamine sulfate ($p<0.05$ vs. placebo), with clinically significant improvement being evident beginning in the second week and continuing thereafter. The responder rate after 4 weeks (calculated as a decrease of at least 3 points in the Lequesne index, together with a positive overall judgment by the investigator) indicated 52 percent of patients responded to crystalline glucosamine sulfate while 37 percent responded to the placebo, in the intent-to-treat population of 126 patients in each group ($p=0.016$).

Tolerability was good and similar between crystalline glucosamine sulfate and placebo, with a six percent incidence of minor adverse events in the crystalline glucosamine sulfate group and ten percent in the placebo group (with four percent and six percent related drop-out incidence, respectively). Routine laboratory tests at entry and study completion did not show any clinically significant modifications.

ii. Müller Fassbender H et al. Osteoarthritis Cart 1994; 2:61-69 – Reference no. 33)

In a twin study to the Noack study (32), Müller et. al. evaluated the same crystalline glucosamine sulfate dosage with ibuprofen at the standard analgesic dose of 1200 mg/day, for 4 weeks. Two hundred patients from a rehabilitation clinic, with knee osteoarthritis and clinical signs of joint flare, were randomized to the active treatments. The Lequesne index (modified by duplicating the pain scores to take into account the bilateral knee involvement in most of the patients) indicated a slightly more severe impairment as compared to the Noack study (32). Nevertheless, no rescue treatment was allowed, with the only exception of a concomitant physical therapy program, for which there were no differences between the two groups.

The responder rate, calculated with a method similar to that of the Noack study (32), was around 50 percent in both groups (48 percent with crystalline glucosamine sulfate and 52 percent with ibuprofen; p=0.67), with a ~40 percent reduction in the Lequesne index after 4 weeks. Consistent with the results reported in earlier studies, the development of the symptomatic effect differed between the two treatments. Ibuprofen induced a faster, although not statistically significant, symptom relief concentrated in the first two weeks while crystalline glucosamine sulfate induced a slower but constant

improvement that was superimposable to that of the NSAIDS from the third week onward.

Crystalline glucosamine sulfate was significantly better tolerated than ibuprofen, with adverse events reported in six percent of patients compared with 35 percent with ibuprofen ($p<0.001$). A related discontinuation rate of only one percent was reported for crystalline glucosamine sulfate while the ibuprofen group had a seven percent rate ($p=0.035$). Adverse events were mainly gastrointestinal in nature, as expected with the mixed COX_1-COX_2 inhibitor ibuprofen. No clinically significant laboratory changes were observed.

iii. Qiu GX et al. Arzneimittelforschung 1998 ; 48 : 469-74 (Reference n. 34)

Qiu also conducted a randomized, double-blind study that compared crystalline glucosamine sulfate (1500 mg glucosamine sulfate/day) with ibuprofen (1200 mg ibuprofen/day). This study confirmed the short-term symptomatic effect of crystalline glucosamine sulfate in knee osteoarthritis in an ethnically distinct population (178 Chinese patients). Knee pain improved with both treatments throughout the four weeks of treatment, with a non-statistically significant difference in favor of crystalline glucosamine sulfate, sustained for the two weeks of follow-up after treatment withdrawal. Also in this case, adverse reactions and related drop-outs were lower with crystalline glucosamine sulfate compared with ibuprofen ($p=0.02$ and $p=0.0017$, respectively).

b. **Long-term pivotal trials**

i. Reginster JY et al. Lancet 2001; 357:251-56 (Reference n.15)

Reginster et al. examined the long-term efficacy and safety of crystalline glucosamine sulfate on the risk of progression of osteoarthritis. A total of 212 patients (mean age 66 years; 76 percent females) with knee osteoarthritis (diagnosed using American College of Rheumatology (ACR) criteria) were randomized to continuous oral treatment with crystalline glucosamine sulfate (equivalent to 1500 mg glucosamine sulfate) once per day or placebo, for three years in a double-blind fashion. Disease severity in terms of symptoms and joint structure changes was mild to moderate. After three years, symptoms had improved to a significantly larger extent in the crystalline glucosamine sulfate group compared with placebo, as evaluated on the WOMAC index subscales of pain and joint function. Percent changes on the global index indicated a ~10 percent worsening with placebo and an improvement with crystalline glucosamine sulfate that was significantly different in both the intention-to-treat population and the per-protocol completers, and as high as 25 percent in the latter.

This study also detected for the first time, a significant joint structure-modifying effect. The primary structural end-point was joint space narrowing evaluated by digital image analysis of the mean joint space width of the medial tibiofemoral joint compartment, as well as by visual inspection (with the aid of a magnifying glass) at the joint's narrowest point. Standardized weight-bearing, antero-posterior radiographs of each knee in full extension were taken at enrollment and after one and three years according to state-of-the-art methodology at the time of study design. Placebo-treated patients suffered a mean joint space narrowing of approximately –0.1 mm/year, which is in line with the structural progression reported in the literature for knee osteoarthritis.

No narrowing occurred on average in the crystalline glucosamine sulfate group and the final differences between groups were significant. Furthermore, 30 percent of patients randomized to placebo presented a severe mean joint space narrowing of more than 0.5 mm, which may predict disability in the future, compared with only 15 percent of patients on crystalline glucosamine sulfate ($p = 0.013$).

There were no significant differences between crystalline glucosamine sulfate and placebo in frequency or pattern of adverse events. Laboratory tests did not show significant abnormalities on system organs or metabolic functions.

The authors of this report openly cautioned against generalizing the results of this study to other sources of glucosamine. Reginster states "[i]n this study glucosamine sulphate was approved as a prescription drug, therefore, our results cannot be generalised to other glucosamine products (or compound mixtures) such as those available in some countries as dietary supplements" (15). In the accompanying editorial to the Reginster study, Dr. Tim McAlindon, Arthritis Center Boston University Medical Center and the principal author of the JAMA meta-analysis (17) notes "since glucosamine is generally self-prescribed, the likely primary beneficiary of this trial will be the nutritional-product industry rather than the pharmaceutical company that sponsored the trial, even though the results may not be generalisable to the highly variable formulations of nutritional products" (35).

In his editorial comments, McAlindon also defined glucosamine for osteoarthritis as the possible dawn of a new era in the treatment of this disease and characterized the Reginster trial as a landmark study in osteoarthritis research (35).

Bruyere et al. published two full reports derived from the trial of Reginster. In the first report (36), a poor correlation between symptoms and radiographic joint structure parameters was observed, as widely described in the scientific literature about osteoarthritis progression. There was, however, a modest significant correlation between knee pain and joint space narrowing. Interestingly, the symptom-modifying effect of crystalline glucosamine sulfate was significantly independent of baseline joint structural damage and its progression. The latter was described in the second report (37): patients with better-preserved joint structure at baseline suffered the most dramatic joint space narrowing after three years when receiving placebo and were those in which the structure-modifying effect of glucosamine was more evident.

ii. Pavelka K et al. Arch Int Med 2002 ;162 : 2113-23 (IV.B.15 – Reference n.16)

This trial independently confirmed the results of Reginster (15) with an almost identical protocol and patient population (202 knee osteoarthritis patients; mean age 62; 78 percent female). Throughout the three years of treatment, symptoms steadily improved with crystalline glucosamine sulfate on the Lequesne index (from mild to moderate baseline values of 8-9 points) over the first year of treatment. This improvement remained constant until the end of the study, with a pattern that differed significantly from placebo. After 3 years, reduction in pain, function limitation, and stiffness was significant for crystalline glucosamine sulfate compared with placebo on the WOMAC index and its subscales, and on the overall Lequesne index, with an effect size that was similar to that observed in the study by Reginster.

This study also reported a striking structure-modifying activity obtained with the same conventional method and similar effect size reported by Reginster (15). Pavelka

reported a progressive joint space narrowing with placebo at each treatment year, while no average loss in joint space width occurred in glucosamine-treated patients. The authors report that 14 percent of the patients on the placebo lost over 0.5 mm joint space width while only five percent receiving crystalline glucosamine sulfate lost 0.5 mm of joint space width ($p=0.05$). The authors performed an analysis of secondary radiographic features of osteoarthritis and reported a three-fold higher proportion of worsening osteophyte scores (according to a validated radiographic atlas) in the placebo group when compared to the crystalline glucosamine sulfate group. Safety was again similar between crystalline glucosamine sulfate and placebo and is described in detail in the publication.

As did Reginster, Pavelka warns against generalization of the results to other glucosamine preparations other than this original crystalline glucosamine sulfate formulation. Pavelka states

> Glucosamine derivatives are popular dietary supplements in the United States and other countries, exploiting the opportunity provided by the American Dietary Supplement Health and Education Act and the clinical research data obtained with glucosamine sulfate approved as a prescription drug for the treatment of osteoarthritis in Europe and elsewhere. The latter was used in our study and in most of the previous clinical experiences; at present, it is difficult to generalize these results to the highly variable and uncontrolled formulations of the other nutritional products claiming a glucosamine content.

It is interesting to note that the structure-modification findings reported in the Reginster and the Pavelka studies have been questioned because of the use of the conventional standing (full extension) knee radiographic view (38). It has been hypothesized that the major pain relief in the crystalline glucosamine sulfate arm relative to placebo altered the positioning of the knee (favoring a better knee full extension) that might have confounded the estimate of joint space narrowing and exaggerated the differences between treatment groups. The conventional standing (full extension) knee radiographic view was the gold standard at the time of the study design and recommended in scientific guidelines. None of the more recently proposed techniques, such as the new semi-flexed views, have been validated in longitudinal studies (38). Moreover, although this criticism acknowledges the potent and previously unseen (with any other agent) long-term symptom-modifying effect of crystalline glucosamine sulfate, the authors of the two glucosamine long-term trials have elegantly shown that pain relief did not confound the assessment of joint space narrowing. The authors, therefore, have adequately validated their results with respect to structure modification, first in the Pavelka report (published in full after these criticisms were raised) and then in a joint abstract (39).

c. <u>Other recent clinical studies</u>

Two additional studies (40, 41) were included in one or more of the Cochrane (20), JAMA (17), or Richy (22) review articles and meta-analyses, but cannot be considered pivotal for the reasons expressed below. A third study by Förster et al. (42) was not included in any of the meta-analyses, but offers corroborative evidence of the safety and efficacy of crystalline glucosamine sulfate.

Reichelt et al. (40) performed a randomized, placebo-controlled, double-blind trial of glucosamine for six weeks using an intramuscular injection of crystalline glucosamine sulfate. The patients received two injections per week of 400 mg of glucosamine sulfate. This study has limited relevance to the dietary supplementation of crystalline glucosamine sulfate because it involves a different route of administration. Nevertheless, the study is sufficiently large (155 patients with knee osteoarthritis) and well conducted. The authors report a significant decrease in the Lequesne index compared with placebo over the 6 weeks of treatment, which was of comparable magnitude to that observed with oral crystalline glucosamine sulfate. The authors also report that intramuscular injection of crystalline glucosamine sulfate had comparable safety to the placebo.

The second study is of limited value because it is published only in abstract form (41) and is not widely publicly available as a full report. This report is, nonetheless, reviewed in the three meta-analyses (17,20,22) (with very high quality scores in the latter two) and is therefore included here as corroborative evidence. Further information about the study is publicly available from a product monograph (43). In this double-blind study, 319 patients with knee osteoarthritis and moderate to severe symptoms (with baseline Lequesne index mean values around 10-11 points and pain visual analogue scores ≥40 mm out of 100) were randomized to oral crystalline glucosamine sulfate equivalent to 1500 mg glucosamine sulfate once daily), or the conventional NSAID piroxicam (20 mg/day), or the combination of the two agents, or placebo. Treatment was administered for 12 weeks, followed by an eight-week observation period without treatment.

After 4 weeks of treatment, the crystalline glucosamine sulfate symptom-modifying effects were virtually superimposable to those previously described, with a decrease in the Lequesne index of around 3 points in average, similar to that achieved with piroxicam. Thereafter, the improvement continued almost linearly with crystalline glucosamine sulfate. The average decrease exceeded 4 points at the end of the 12 weeks, while it remained around 3 points with piroxicam: both groups displayed significantly better effects than placebo ($p<0.001$), but crystalline glucosamine sulfate tended to be better than the NSAID ($p<0.05$). After withdrawal of the agent administration, the crystalline glucosamine sulfate symptomatic effect was sustained for the 8-week follow-up ($p<0.01$ vs placebo), while the effect was rapidly lost with the piroxicam group. Combination of crystalline glucosamine sulfate with the NSAID tended to show a faster symptom relief over the first 2-4 weeks than each separate agent, but it was afterwards superimposable to crystalline glucosamine sulfate alone.

Patients receiving crystalline glucosamine sulfate had a similar incidence of adverse events than those taking placebo, but significantly less than those in the piroxicam group (15 percent vs 42 percent, $p<0.001$, with 9 percent vs. 33 percent referred to the gastrointestinal tract) with fewer drop outs. Combination of crystalline glucosamine sulfate with the NSAID did not prevent the adverse reactions from the latter.

Finally, Förster et al. recently published, although only in abstract form (42), a randomized, placebo-controlled, double-blind study of the standard crystalline glucosamine sulfate once-daily formulation and dosage, in 160 patients with osteoarthritis of the spine. The substance induced a significant improvement vs.

placebo in most of the pain and functional parameters evaluated over the 6-week treatment course, which was sustained for the 4-week follow-up without treatment. The improvements were apparently more marked in the lumbar spine compared with the cervical spine (not reported). There were no differences between glucosamine and placebo with respect to safety.

3. Clinical trials performed with glucosamine formulations other than crystalline glucosamine sulfate

There are only a few published clinical trials that have evaluated sources of glucosamine other than crystalline glucosamine sulfate. The Petitioner is unaware of any unpublished studies on these other sources of glucosamine. The few published studies that are available, and that are reviewed below, have failed to yield the same consistent results that have been reported in the trials involving crystalline glucosamine sulfate.

Houpt et al. (44) performed a study with glucosamine hydrochloride that is taken into consideration in both the JAMA and Cochrane reviews (but not in the meta-analysis of Richy et al., which deals only with glucosamine sulfate). A total of 101 patients with knee osteoarthritis were randomized and dispensed either glucosamine (as hydrochloride, at a total daily dose of 1500 mg) or placebo for 8 weeks. Houpt used the WOMAC algo-functional index as a primary measure of outcome. With one exception, scores on the remaining 23 WOMAC questions tended to improve with glucosamine compared with placebo. The degree of improvement tended to be greater in the glucosamine group vs. placebo in all WOMAC subscales of pain, function, and stiffness (~20 percent improvement with glucosamine vs less than 10 percent with placebo). However, the difference between groups in the WOMAC score changes failed to reach

statistical significance; a patient daily diary for pain showed a statistically significant pain reduction for glucosamine vs. placebo at some points during the study. Safety was similar between glucosamine and placebo, with 12 percent of subjects in both groups reporting mild gastrointestinal symptom (bloating or cramps).

As discussed in the Cochrane Review, it seems that differences in efficacy may relate to differences between glucosamine sulfate (in appropriate formulations) and hydrochloride salts. As acknowledged by Houpt in their introduction, the efficacy of crystalline glucosamine sulfate is well established while that of glucosamine hydrochloride is only anecdotal. Although detecting a trend for efficacy for glucosamine, the study by Houpt seems to indicate that there may be major differences between the crystalline glucosamine sulfate and the hydrochloride salt that would discourage the translation of efficacy results obtained with the former to preparations containing the latter substance.

Rindone et al. (23) published a small (98 patients), randomized, placebo-controlled, double-blind, parallel-group, 8-week study using an unspecified oral formulation and salt of glucosamine (although the study is considered in the meta-analysis by Richy as being performed with a glucosamine sulfate formulation). To further challenge the poor statistical power of the study, the authors enrolled patients in apparently more severe conditions than in other short-term studies with crystalline glucosamine sulfate. As widely discussed in the paper to explain the study's many limitations, the patients had longer-standing disease, were only men, were heavier, had much more severe disease from the point of view of radiographic staging and, above all, were unresponsive to conventional symptomatic medications (60 percent of patients

were taking NSAIDs or analgesics and were asked not to discontinue them). There were no significant differences between glucosamine and placebo on the symptom evaluations performed. Thirty-four percent of patients taking glucosamine reported mild and self-limiting effects vs. 23 percent with placebo (2 related withdrawals with glucosamine vs. 4 with placebo).

Hughes and Carr (24) recently published a very small (40 patients/group) 6-month, randomized, double-blind, placebo-controlled study of a glucosamine formulation (potassium chloride glucosamine sulfate, plus vitamin C, calcium carbonate and manganese) not tested previously in any clinical trial to the knowledge of the Petitioner. The study was considered in the recent meta-analysis by Richy (22). The authors described the trial design as pragmatic in that they enrolled patients with a wide range of pain and other symptom severity and included all grades of radiological severity, which is contrary to the standard practice in osteoarthritis clinical trials. The patient population varied in terms of osteoarthritis severity with 10 percent of the population classified as a Kellgren and Lawrence radiological grade 1 (doubtful osteoarthritis), and over 20 percent presented with a grade 4 (severe osteoarthritis); patients on these extreme ends of the Kellgren and Lawrence radiological grade are usually excluded from clinical trials. To further complicate matters, almost 50 percent of patients were on NSAIDs and were asked not to discontinue them, making it difficult to assess the treatment efficacy and the rescue analgesic consumption. Indeed, there were no significant differences between glucosamine and placebo in any of the validated symptom end-points of the study. The placebo effect was classified to be strong by the authors. Indeed the authors report elsewhere that most of the patients misunderstood the study information and thought that everyone in the study was

receiving glucosamine (45), which might have seriously biased the study. It is very difficult to draw any conclusion on the efficacy of this glucosamine sulfate preparation based on this trial. Also in this case, safety was similar to that of placebo.

In a preliminary study, Thie et al. (46) described a similar efficacy between a glucosamine sulfate formulation and ibuprofen in a small group of patients with osteoarthritis of the temporomandibular joint.

In a small (34 patients with degenerative joint disease), 16-week, randomized, placebo-controlled, cross-over trial, Leffler et al. (47) reported a significant benefit on knee osteoarthritis symptoms with a combination of glucosamine hydrochloride, chondroitin sulfate, and manganese ascorbate. Similar results were recently described by Das and Hammad (48) in a slightly larger group (93 patients only) of knee osteoarthritis subjects treated for 6 months. Although these are two favorable studies, there may be reservations on the relevance of such a small sample size for generalizability of the results. In addition, caution appears to be in order due to the likely metabolism of chondroitin sulfate.

Chondroitin sulfate is a glycosaminoglycan normally present in the cartilage matrix and consisting of a high molecular weight, long chain of repeating units of differently sulfated residues of glucuronic acid and N-acetyl galactosamine, obtained with extraction processes from animal tissues. It is used in some countries for the treatment of osteoarthritis under the rationale that it is speculatively similar to that of glucosamine sulfate. The rationale is difficult to understand given the major differences in physico-chemical properties between the two (*i.e.*, a macromolecular tissue extract

compared to a low molecular weight pure glucosamine sulfate). Actually, oral absorption of high molecular mass polymers is questionable. Pharmacokinetic studies (49) have shown that after oral administration the largest peak consists of one of the constituent monomers, N-acetyl-galactosamine, which is probably responsible for the beneficial activity, although absorption of a small fraction of high molecular weight chondroitin sulfate cannot be excluded. Very early studies had shown that N-acetyl-galactosamine might induce metabolic activities similar to that of its precursor glucosamine, although with a lower potency (50). It may be speculated, therefore, that the clinical activity reported for chondroitin sulfate in some clinical trials may be similar to that of low dose glucosamine sulfate.

Based on anecdotal evidence, it is sometimes claimed that a combination of dietary supplements containing chondroitin sulfate and glucosamine-derivatives may offer added value in the treatment of osteoarthritis. However, there is no scientific proof for this claim and, on the basis of the discussion above, the rationale of such a combination is also weak because adding chondroitin to glucosamine would presumably only slightly increase the dose of glucosamine. The two very small clinical trials reviewed here (47,48) do not allow one to distinguish between the effects of glucosamine alone or of the combination. It seems, therefore, that combinations of glucosamine and chondroitin sulfate cannot benefit from the claims proposed for crystalline glucosamine sulfate.

C. Level of crystalline glucosamine sulfate needed to justify the claim

As is described in the above summary of scientific evidence, the clinical studies consistently used 1500 mg/day of glucosamine sulfate, corresponding to 1884 mg of

crystalline glucosamine sulfate. The Petitioner considers this to be the optimum level of intake beyond which the health benefits of crystalline glucosamine sulfate have not been demonstrated clinically and thus are not to be expected. The Petitioner is unaware of any level at which an adverse effect from crystalline glucosamine sulfate would be expected to occur. The Petitioner is aware that certain populations, namely, individuals with diabetes, may need to receive careful monitoring while taking crystalline glucosamine sulfate, as it has been suggested—but not established—that crystalline glucosamine sulfate may increase insulin resistance.

In the United States, crystalline glucosamine sulfate is available as caplets of 750 mg glucosamine sulfate and as sachets of powder for oral solution, dosed for once-daily administration of 1884 mg crystalline glucosamine sulfate (1500 mg glucosamine sulfate). This latter formulation is particularly relevant because it is the formulation used in the latest clinical trials, including the long-term studies.

D. Global analysis of safety

The safety of glucosamine for use as a dietary supplement is supported by the numerous clinical trials, by its physical properties, chemical structure, and metabolic fate, and by experience based on widespread use in the United States and throughout the world. The safety of crystalline glucosamine sulfate is also acknowledged by the three systematic reviews and meta-analyses that have published.

1. Clinical trial data

Several recent clinical trials and the meta-analysis publications in prestigious clinical journals (e.g., *Lancet, Journal of the American Medical Association, Archives of Internal Medicine, Cochrane Library*) provide a coherent scientific assessment about

the efficacy and safety of glucosamine. These publications reflect agreement in the scientific community that glucosamine supplementation presents no significant or unreasonable risk of illness or injury.

In the pivotal short-term clinical trials for crystalline glucosamine sulfate (32,33,34), the incidence of patients reporting adverse events on glucosamine ranged between six and 12 percent with drop-outs due to adverse events in less than four percent of the patients. Importantly, none of the studies reported statistically or clinically significant differences between crystalline glucosamine sulfate and placebo in the incidence of adverse events or safety related drop-outs. In the trials comparing crystalline glucosamine sulfate with NSAIDs, the NSAID groups consistently had a significantly higher incidence of adverse events and safety related drop-outs than the group receiving crystalline glucosame sulfate. In those rare instances when patients reported adverse events, they generally involved mild and transient reactions associated with the gastrointestinal system such as abdominal pain or discomfort, nausea, diarrhea, constipation, and/or meteorism. Other reported adverse events include headache, dizziness and minor allergic reactions such as cutaneous rashes with erythema and itching. This pattern of adverse events was similar between crystalline glucosamine sulfate and placebo in all trials.

The pattern of adverse events described in the short-term pivotal clinical trials is similar to that described in all of the other short-term trials (40,41,42,43). No study reported more than 15 percent of patients in the glucosamine groups experiencing adverse events.

The long-term pivotal trials are particularly important, as they allow the assessment of continued safety during prolonged use, which is seldom, if ever, described for agents used in osteoarthritis. In both the Reginster (15) and the Pavelka (16) trials, the safety of crystalline glucosamine sulfate was similar to that of placebo. Because these studies involved long-term administration of crystalline glucosamine sulfate in elderly patients, it is not surprising that most of the patients in the Reginster study reported at least one adverse event with crystalline glucosamine sulfate or placebo, 94 percent and 93 percent, respectively. The patients in the Pavelka study had a slightly lower rate of adverse events in the crystalline glucosamine sulfate and placebo groups of 66 and 64 percent, respectively. The adverse events, however, involved mild to moderate and transient events in the crystalline glucosamine sulfate and placebo groups consisting of abdominal discomfort or pain, dyspepsia and nausea, or disturbed defecation. Musculoskeletal adverse events were likely related to osteoarthritis; treatment-unrelated cardiovascular events were also common in this elderly population, as were urinary infections and seasonal respiratory tract infections. Few and sporadic serious events were also reported in both the crystalline glucosamine sulfate and placebo groups, with no "signal" for any toxicity being detected with the active treatment.

In all short-term and long-term clinical trials, there were no modifications of routine laboratory tests during treatment with crystalline glucosamine sulfate.

2. <u>Properties and Metabolic Fate</u>

The mechanism of action of glucosamine, briefly reviewed in section II of this Petition, does not account for any particular toxicity pattern of crystalline glucosamine

sulfate. Unlike NSAIDs, glucosamine does not inhibit type 1 cyclooxygenase (COX-1), which explains the significantly better safety pattern at the gastrointestinal level.

Osteoarthritis is a chronic disease that involves a majority of elderly patients, who frequently receive treatments for concomitant diseases. Glucosamine does not compete for absorption mechanisms and, after absorption, does not bind to plasma proteins. The metabolic fate of this endogenous substance is mainly the incorporation in proteoglycans or degradation independently of the cytochrome enzyme system. The physicochemical, pharmacokinetic, and metabolic properties of glucosamine suggest a low potential for adverse effects and a low potential for drug interactions. With regard to the latter, the clinical studies have found no evidence of any drug interactions.

Recent animal experimental studies with suprapharmacological intravenous doses of glucosamine suggested that the compound might increase insulin resistance through a complex interaction with the so-called hexosamine pathway (one of the alternative routes of glucose metabolism) (51). Two recent human studies using extremely high intravenous (52) or even intraarterial (53) glucosamine doses indicated that such a mechanism is probably not operating in humans. These studies did not detect an effect on insulin sensitivity, secretion or action by glucosamine administration. Indeed, a recent report (54) clarified that fasting plasma glucose levels were not modified by the short-term crystalline glucosamine sulfate administration, even in patients with hyperglycaemia at baseline, nor in the long-term pivotal trial by Reginster (15), where fasting blood glucose even tended to decrease, on average. In the long-term study by Pavelka (16), four patients developed diabetes during the study, but three were on placebo and only one on crystalline glucosamine sulfate.

A very recent report (55) evaluated a small group of 26 elderly patients with type 2 diabetes, receiving daily supplementation for 90 days with a combination of 1500 mg glucosamine hydrochloride and 1200 mg chondroitin sulfate or placebo (in a randomized, double-blind setting). No patient had any change in their diabetes management during the study. There were no significant differences between groups in post-treatment haemoglobin A_{1c} concentrations, nor were there any significant differences within groups before and after treatment. Although this report is reassuring, given the differences between crystalline glucosamine sulfate and glucosamine hydrochloride, the results of this study should not be generalized to crystalline glucosamine sulfate.

The clinical studies published to date have failed to report any findings establishing concern with the administration of crystalline glucosamine sulfate to diabetics. The data in this patient population, however, is recognizably relatively scarce.

3. <u>Experience based on use</u>

The extensive use of glucosamine products throughout the world also supports its safe use. In the United States, glucosamine products are among the most widely marketed dietary supplements. In one recent U.S. market analysis, glucosamine was ranked as the 11th best selling supplement in the United States; in a dietary supplement use survey, glucosamine was ranked as the fourth most common supplement among a randomly selected sample of 2590 adult men and women (National Academy of Sciences, Institute of Medicine, Safety Review: Draft Prototype Monograph on Glucosamine (Jan. 2003)). Although widely consumed as a dietary supplement, the

published literature contains few reports of adverse events. Adverse events that are attributed to glucosamine are generally mild and transient, and often relate to gastrointestinal concerns.

Although crystalline glucosamine sulfate is regulated as a dietary supplement in the United States, it has been available as a prescription drug in over 40 countries of the world for over two decades. Among the countries in which glucosamine is regulated as a drug are seven countries within the European Community-EC (Finland, Germany, Greece, Ireland, Italy, Portugal and Spain) and several other European countries (Baltic countries, Bulgaria, Czech Republic, Hungary, Poland, Rumania, Russia, Slovak Republic). The substance has been extensively used in practice (as evident from market data) on a wide geographical basis throughout the world, but no safety issues have been raised through the pharmacovigilance monitoring system in Europe and in other countries where the substance has a prescription drug status.

IV. HIGH RANKING FOR THE STRENGTH OF THE EVIDENCE FOR THE PROPOSED HEALTH CLAIM FOR CRYSTALLINE GLUCOSAMINE SULFATE

The Petitioner has evaluated the strength of the total body of evidence that supports the proposed claim for crystalline glucosamine sulfate according to the FDA guidance regarding significant scientific agreement (*Guidance for Industry: Significant Scientific Agreement in the Review of Health Claims for Conventional Foods and Dietary Supplements*) and recent guidelines concerning the review of so-called "qualified health claims" (*Interim Evidence-based Ranking System for Scientific Data*). In the opinion of the Petitioner, available evidence reflects significant scientific agreement among qualified experts that crystalline glucosamine sulfate reduces the risk of osteoarthritis-related joint deterioration, joint pain, and limitation of function. Should FDA disagree, however, the Petitioner would be willing to consider review of the proposed claim as a "qualified health claim," as described in agency guidance of July 11, 2003. In light of the extensive research to date addressing the relationship between crystalline glucosamine sulfate and osteoarthritis, the proposed claim is, at a minimum, a "Category B" claim for which the scientific evidence may be described as supportive but not conclusive.

The clinical studies convincingly establish that an oral dose of crystalline glucosamine sulfate (1500 mg of glucosamine sulfate/day) is an effective therapy for patients with mild, moderate and even severe osteoarthritis by reducing joint deterioration and accompanying joint pain and limitation of function. Indeed, existing clinical practice guidelines for osteoarthritis published by major scientific organizations support the use of crystalline glucosamine sulfate in osteoarthritis. The two most

important recommendations are those released by the American College of Rheumatology (ACR) and by the European League Against Rheumatism (EULAR). These organizations published their most recent guidelines in 2000, (56, 4), prior to the publication of the Cochrane review (20), the meta-analysis by Richy (22) and the long-term pivotal trials by Reginster (15) and by Pavelka (16).

The 2000 EULAR guidelines (4), in their evidence-based assessment, support the short-term efficacy of glucosamine on pain reduction and improved function. The guidelines acknowledge the potential for disease modification by placing crystalline glucosamine sulfate in category 1B—indicating a "high category of evidence" in support of the recommendation. The highest category of evidence is 1A and at the time of the 2000 publication, the EULAR placed only NSAIDs and patient education in the 1A category. The EULAR has since evaluated the new data on crystalline glucosamine sulfate and has updated its practice guidelines (Osteoarthritis Task Force: EULAR recommendations for treatment of knee osteoarthritis – 2003 Version, presented during the Annual European Congress of Rheumatology – Lisbon, Portugal, 18-21 June 2003 – Full report publication due in the fall of 2003). Glucosamine sulfate has now been placed in category 1A indicating that there is the highest level of evidence with a very large effect size and very high quality clinical trials supporting its efficacy in this patient population.

A four-member ACR subcommittee issued a consensus guideline in 2000 (56) that recognized glucosamine among the agents under investigation and stated that "while a number of studies support the efficacy . . . for palliation of joint pain . . . the subcommittee believes that it is premature to make specific recommendations . . . ."

The ACR has not yet updated these guidelines to consider the compelling data on crystalline glucosamine sulfate published since this earlier review. Hochberg, one of the four members of the ACR subcommittee did publish an "informal update" of the ACR guidelines in 2001 with a paper entitled "What a difference a year makes: reflections on the ACR recommendations for the medical management of osteoarthritis" (57). In this paper, Hochberg reviews the new evidence appearing in the year following the ACR guideline publication that stress, among others, the recommendation for the use of glucosamine. Hochberg acknowledges the different evidence-based perspective of the EULAR guidelines and, especially the appearance in early 2001 of the glucosamine Cochrane Review and the long-term trial by Reginster. With a particular emphasis on the original crystalline glucosamine sulfate preparation, Hochberg's analysis encourages the U.S. medical community "to reassess the use of glucosamine as a first-line agent at least for patients with knee osteoarthritis who have mild-to-moderate pain".

Hochberg's focus on patients with mild-to-moderate osteoarthritis derives from the emphasis placed on Reginster's long-term study, later reinforced by Pavelka's study. Although this focus is definitely correct for the reduction in the risk of osteoarthritis joint structure deterioration, Reginster's group has shown that symptoms are relieved by glucosamine irrespectively of baseline disease severity. In addition, the short-term trials reviewed here indicate the symptomatic effect of crystalline glucosamine sulfate also in moderate-to-severe patients.

The slightly different recommendations coming out of these European and U.S. expert bodies is not surprising. There are major differences in the perception of the role

of glucosamine for osteoarthritis in the United States and in Europe. While crystalline glucosamine sulfate is regulated in much of Europe as a prescription drug, it and other forms of glucosamine are regulated as a dietary supplement in the United States. The tepid recommendation from the ACR consensus guideline may very well reflect the concern in the U.S. medical community that patients with osteoarthritis will try to self-medicate without first seeking the intervention of a medical professional. In addition, most patients lack the sophistication to distinguish the various sources of glucosamine and may chose a formulation other than crystalline glucosamine sulfate which is the standardized formulation with robust clinical data supportive of its use. Regardless of the reason for the different recommendations, Hochberg's "informal update" of the 2000 ACR consensus guidelines does recommend the use of crystalline glucosamine sulfate in osteoarthritis patients with mild to moderate pain.

While we recognize that data in this Petition convincingly establish that crystalline glucosamine sulfate has been proven to be an effective therapy for individuals with osteoarthritis, the data also establish that crystalline glucosamine sulfate is effective at reducing the risk of developing osteoarthritis joint structure degradation, pain, and limitation of function. FDA has previously recognized that it is appropriate to consider clinical studies involving a diseased population to support a health claim for a reduced risk of developing a disease. Perhaps most relevant are the extensive data supporting the cardiovascular benefits of omega-3 fatty acids in patients with cardiovascular disease.

When issuing the qualified health claim for dietary supplements of omega-3 fatty acids, the agency specifically recognized that "the evidence from interventional

trials with CHD as an endpoint is strongly favourable in a diseased population showing that omega-3 fatty acid intake is related to reduced risk of CHD" and "that there is suggestive evidence that the benefit on CHD reported in diseased populations will carry over to the general population because omega-3 fatty acids have similar physiological effects in both diseased and general populations" (Letter from Christine J. Lewis, Ph.D., to Jonathan W. Emord, Esq. (Oct. 31, 2000)). Although the agency cited the absence of controlled studies in the healthy population as one of the factors preventing a finding of significant scientific agreement for omega-3 fatty acids, the Petitioner believes that the clinical studies on crystalline glucosamine sulfate provide an even more compelling case than those studies with omega-3 fatty acids.

The physiological effect of crystalline glucosamine sulfate is well characterized. Crystalline glucosamine sulfate not only provides one of the building blocks for cartilage synthesis, but it suppresses those enzymes, that in patients with osteoarthritis, create an imbalance by breaking down cartilage at a faster rate than it is synthesized. Crystalline glucosamine sulfate will have the same physiological impact in the healthy population, as determined by clinical studies of patients with "mild osteoarthritis," a patient population quite similar to the general population.

The clinical studies are supportive of the use of crystalline glucosamine sulfate in osteoarthritis prevention, particularly those studies that evaluated patients with a mild form of the disease. Indeed, Hochberg (57) specifically recommends the use of crystalline glucosamine sulfate in patients with mild osteoarthritis. The long-term study of Reginster (15) further supports the ability of crystalline glucosamine sulfate to reduce the risk of developing osteoarthritis. Reginster demonstrated that the protective effects

on joint structure are dramatic in those patients that started the trial with a better-preserved joint (37). Reginster classified certain patients as having "mild osteoarthritis" based on the radiographic joint space width at the beginning of the trial. The preventative effects of crystalline glucosamine sulfate in this patient population with "mild osteoarthritis," a patient population very similar to the "healthy population," combined with the well-known mechanism of action for crystalline glucosamine sulfate support the ability of crystalline glucosamine sulfate to be effective in preventing the onset of osteoarthritis.

The Petitioner believes that the evidence in this Petition establishes that there is significant scientific agreement among qualified experts to support the proposed health claim for crystalline glucosamine sulfate (*i.e.*, a "high level of comfort" that is not likely to be reversed by new and emerging evidence). Accordingly, it is the opinion of Petitioner that the proposed claim should be allowed as a First Level Scientific Ranking and FDA Category A health claim. To the extent that FDA disagrees with this assessment, Petitioner would accept classification of the evidence into FDA Category B and the appropriate qualifying language that would need to accompany such a health claim (*i.e.*, "although there is scientific evidence supporting the claim, the evidence is not conclusive").

V. GLUCOSAMINE FORMULATIONS OTHER THAN CRYSTALLINE GLUCOSAMINE SULFATE DO NOT HAVE THE SAME BODY OF EVIDENCE TO SUPPORT ANY CLAIM

Although several forms of glucosamine are presently marketed for dietary supplement use in the United States, crystalline glucosamine sulfate is the only form of glucosamine that has been studied extensively. Crystalline glucosamine sulfate was recently referred to in the USP/NF 2003 as Glucosamine Sulfate Sodium Chloride (crystalline glucosamine sulfate) that, when dissolved in water, gives a solution containing glucosamine, sulfate, sodium and chloride ions in stoichimetric ratios of 2:1:2:2. For simplicity, this specific substance is referred to in the clinical and other scientific literature as glucosamine sulfate. Other forms of glucosamine include glucosamine hydrochloride, or N-acetyl-glucosamine or other, sometimes unspecified "glucosamine sulfate" formulations.

These other forms of glucosamine (*i.e.*, glucosamine hydrochloride, N-acetyl-glucosamine, or other "glucosamine sulfate" formulations) may not share the same quality, pharmacological, pharmacokinetic and, especially, clinical properties of crystalline glucosamine sulfate. The publicly available data establish that the same degree of confidence applicable to crystalline glucosamine sulfate does not apply to any other glucosamine formulations. There are few clinical trials performed with formulations other than crystalline glucosamine sulfate and those studies that have been conducted generally feature small sample sizes with less consistency in results when compared to the studies with crystalline glucosamine sulfate.

The results from the studies on crystalline glucosamine sulfate cannot be generalized to other forms of glucosamine. There are important chemical and physiological differences between the various sources of glucosamine. As stated throughout this Petition, these differences include the following:

- Other sources of glucosamine have not been shown to be bioequivalent with the crystalline glucosamine sulfate formulations used in clinical trials.
- The clinical trials conducted on other sources of glucosamine have yielded less consistent results than those with crystalline glucosamine sulfate.
- The inorganic sulfates found in crystalline glucosamine sulfate are also believed to contribute to its physiological effects. These inorganic sulfates are not found in other sources of glucosamine. Sulfates control the rate of synethsis of the glycosaminoglycan and proteoglycan that become a part of the cartilage matrix. The sulfate serum levels increase after glucosamine sulfate administration (14). The importance of sulfate in the cartilage synthesis process provides further support for using a source of glucosamine that provides sulfates.

The published literature also is replete with statements cautioning against the generalization of the results from studies involving crystalline glucosamine sulfate to other sources of glucosamine. We repeat below many of the concerns that have been expressed in the scientific literature:

- The authors of the Cochrane review (20) noted that all but one of the trials used crystalline glucosamine sulfate and that the one trial with glucosamine hydrochloride gave less favourable, or at least more variable results. The authors conclude that the glucosamine formulation and the presence of sulfates, therefore, seem to be important.
- The meta-analysis peformed by Richey (22) noted that the two studies using other formulations of glucosamine sulfate (23, 24) emerged as those with the lowest effect size.
- Reginster states "[i]n this study glucosamine sulphate was approved as a prescription drug, therefore, our results cannot be generalised to other glucosamine products (or compound mixtures) such as those available in some countries as dietary supplements" (15).
- In the accompanying editorial to the Reginster study, Dr. Tim McAlindon, Arthritis Center Boston University Medical Center and the principal author of the JAMA review (17) notes "since glucosamine is generally self-prescribed, the likely primary beneficiary of this trial will be the nutritional-product industry rather than the pharmaceutical company that sponsored the trial, even though the results may not be generalisable to the highly variable formulations of nutritional products" (35).
- Pavelka (16) states "[g]lucosamine derivatives are popular dietary supplements in the United States and other countries, exploiting the opportunity provided by the American Dietary Supplement Health and Education Act and the clinical research data obtained with glucosamine sulfate approved as a prescription drug for the treatment of osteoarthritis

in Europe and elsewhere. The latter was used in our study and in most of the previous clinical experiences; at present, it is difficult to generalize these results to the highly variable and uncontrolled formulations of the other nutritional products claiming a glucosamine content."

- Houpt (44) notes in the introductory remarks on his study of glucosamine hydrochloride that efficacy of crystalline glucosamine sulfate is well established, while that of glucosamine hydrochloride is only anecdotal.

Given the concerns expressed in the scientific community and the differences between crystalline glucosamine sulfate and other forms of glucosamine, it simply is not possible to generalize the findings in the crystalline glucosamine sulfate clinical studies to other sources of glucosamine.

Limitation of the proposed health claim to crystalline glucosamine sulfate is consistent with the accepted principle that health claim eligibility must be restricted to the specific substances for which the claimed health benefit has been demonstrated by credible scientific evidence. In the case of health claims regarding specific types of soluble fiber and coronary heart disease (CHD), for example, FDA determined that soluble fiber is a family of heterogeneous substances that differ significantly in their effect on CHD risk. In the final rule that established the health claim regulation (21 C.F.R. § 101.81), the agency decided to authorize the claim for one type of soluble fiber only—beta-glucan from whole oats—for which the available data demonstrated a beneficial effect on CHD risk (62 Fed. Reg. 3583, 3587-88 (Jan. 23, 1997). FDA encouraged manufacturers to Petition for a claim for additional soluble fibers if there was evidence to demonstrate a beneficial effect of such fibers on serum lipid levels and

thus, risk of heart disease. (*Id.*) Indeed, FDA intentionally structured the initial health claim regulation to facilitate its subsequent amendment as evidence became available to support extension of the claim to other sources of soluble fiber. As anticipated, FDA later amended the health claim regulation to include soluble fiber from psyllium seed husk and oatrim—a beta-glucan soluble fiber fraction that is produced from alpha-amylase hydrolyzed oat bran or whole oat flour.

The same case-by-case approach is warranted for health claims regarding crystalline glucosamine sulfate and osteoarthritis risk. As with the soluble fiber, there are physiological differences in the various sources of glucosamine that require the health claim to be specific to each source of glucosamine. Moreover, the precedent established by the soluble fiber health claim establishes that manufacturers of other sources of glucosamine must develop the data and Petition FDA for inclusion of their substance in the health claim. A similar approach is warranted here.

In addition to the absence of clinical studies supporting the efficacy of other sources of glucosamine in the prevention of osteoarthritis, there also is a concern with the variability in glucosamine content of these other products (58). The amount of glucosamine sulfate in the Petitioner's product can be precisely detected by suitable potentiometric methods (Attachment 2). The methods proposed are validated for specificity, linearity, accuracy and precision. Unlike manufacturers of other dietary supplements, the Petitioner does not use the high-performance liquid chromatography method described in the USP 26, NF 21. Surprisingly, this method does not detect "glucosamine" but only the chloride ions present in glucosamine hydrochloride or glucosamine sulfate sodium chloride (crystalline glucosamine sulfate). By using this

USP method it would be possible to detect a content of glucosamine of 100 percent when there is no glucosamine in the dietary supplement (provided, of course, that chloride ions are present in the dietary supplement) (see also Petitioner's comments under Attachment 3).

VI. PROPOSED MODEL CLAIM

The Petitioner believes that the scientific information publicly available and summarized in this Petition, supports the following proposed model health claim claim:

> **Daily dietary supplementation with Crystalline Glucosamine Sulfate reduces the risk of osteoarthritis joint structure deterioration and related joint pain and limitation of function.**

This model claim captures the information presented in this Petition by informing the consumer that crystalline glucosamine sulfate can reduce the risk of osteoarthritis-related joint deterioration, joint pain, and limitation of function. The claim is intended to assist in the prevention of the major health-related conditions that are associated with osteoarthritis.

The Petitioner is willing to consider alternative health claim language that may accurately characterize the nature and weight of the substantiating scientific evidence concerning the benefits of crystalline glucosamine sulfate in osteoarthritis. The claim should not, however, be extended to any other glucosamine formulation or combination product.

VII. POTENTIAL EFFECT OF THE CLAIM ON TOTAL INTAKES OF THE SUBSTANCE

Dietary supplements claiming a glucosamine content are widely used in the United States. Approval of this health claim may further increase the consumption of glucosamine containing supplements, particularly those containing crystalline glucosamine sulfate. The increased consumption of crystalline glucosamine sulfate is expected to have a beneficial impact in that it would reduce the incidence of osteoarthritis. No adverse or beneficial changes in other dietary practices are expected.

VIII. DESCRIPTION OF ATTACHMENTS

All reports from clinical trials that are publicly available are included under Attachment 1, together with all scientific references cited here, in order of appearance in the text of this Petition. To the best of Petitioner's knowledge, all non-clinical studies relied upon in this Petition were conducted in compliance with the good laboratory practices regulations set forth in 21 C.F.R. Part 58 and all clinical studies were either conducted in accordance with the requirements for institutional review set forth at 21 C.F.R. Part 56 or were not subject to such requirements in accordance with 21 C.F.R. § 56.104 or § 56.105, and were conducted in compliance with the requirements for informed consent set forth in 21 C.F.R. Part 50.

Attachment 2 includes the description of the assay of for glucosamine in crystalline glucosamine sulfate.

Attachment 3 includes the Petitioner's comments on the HPLC determination of glucosamine in raw material and tablets described in the USP 26 NF 21 (see section V for Attachments 2 and 3).

Attachment 4 contains the results from a Medline search of the terms "glucosamine and osteoarthritis" and includes articles with and without abstracts. The search has been updated and includes all articles already indexed in Medline at the end of August 2003. Not surprisingly, this search identified numerous publications that have limited relevance for purposes of this Petition. This Petition summarizes only those articles that are of sufficient quality and relevance to provide meaningful input on

the relationship between glucosamine and osteoarthritis. The Petition also includes relevant abstracts that cannot be searched in Medline and other articles related to this topic.

IX. ENVIRONMENTAL IMPACT

Pursuant to 21 C.F.R. § 25.32(p), the requested health claim approval sought in this Petition is categorically excluded from any requirement to prepare an environmental assessment or environmental impact statement.

X. CONCLUSION AND CERTIFICATION

For the foregoing reasons, the Petitioner requests that FDA approves the proposed health claim.

On behalf of Petitioner, and pursuant to 21 C.F.R. § 101.70(h), the undersigned certifies that, to the best of Petitioner's knowledge, the Petition includes all information and views on which Petitioner relies and is a representative and balanced submission that includes all favorable as well as unfavorable information known by Petitioner to be pertinent to the evaluation of the proposed health claim.

Yours very truly,

HOGAN & HARTSON L.L.P.

By_____

Martin J. Hahn
Attorneys for
ROTTA PHARMACEUTICALS, INC.

XI. REFERENCES

1 Felson DT, Lawrence RC, Dieppe PA, et al. Osteoarthritis: new insights. Part 1: the disease and its risk factors. *Ann Intern Med.* 2000;133:635-646.

2 Felson DT, Zhang Y. An update on the epidemiology of knee and hip osteoarthritis with a view to prevention. *Arthritis Rheum.* 1998;41:1343-1355

3 Oliveira SA, Felson DT, Reed JI, Cirillo PA, Walker AM. Incidence of symptomatic hand, hip and knee osteoarthritis among patients in a health maintenance organisation. *Arthritis Rheum.* 1995;38:1134-1141

4 Pendleton A, Arden N, Dougados M, et al. EULAR recommendations for the management of knee osteoarthritis: report of a task force of the Standing Committee for International Clinical Studies Including Therapeutic Trials (ESCISIT). *Ann Rheum Dis.* 2000;59:936-944.

5 Guccione AA, Felson DT, Anderson JJ, et al. The effects of specific medical conditions on the functional limitations of elders in the Framingharn Study. *Am J Public Health.* 1994;84:351-358

6 Hamerman D. The biology of osteoarthritis. N Engl J Med 1989; 320:1322-1330

7 Setnikar I, Rovati LC. Absorption, distribution, metabolism and excretion of glucosamine sulfate: a review. *Arzneimittelforschung* 2001; 51: 699-725

8 Noyszewski EA, Wroblewski K, Dodge GR, et al. Preferential incorporation of glucosamine into the galactosamine moieties of chondroitin sulfates in articular cartilage explants. *Arthritis Rheum* 2001;44:1089-1095

9 Bassleer C, Rovati LC, Franchimont P. Glucosamine sulfate stimulates proteoglycan production in human chondrocytes in vitro. *Osteoarthritis Cartilage* 1998;6:427-434

10 Piperno M, Reboul P, Helio Le Graverand MP, et al. Glucosamine sulfate modulates dysregulated activities of human osteoarthritic chondrocytes in vitro. *Osteoarthritis Cartilage* 2000;8:207-212

11 Dodge GR, Jimenez SA, Glucosamine sulfate modulates the levels of aggrecan and matrix metalloproteinase-3 synthesized by cultured human osteoarthritis articular chondrocytes, *Osteoarthritis Cartilage* 2003;11,424-432

12 Yaron I, Shirazi I, Judovich R, Yaron M. Glucosamine sulfate inhibits nitric oxide and stromelysin production in cartilage cultures and reverses IL-1 inhibition of osteoarthritic articular cartilage synthesis. *Ann Rheum Dis* 2001;60 (Suppl.):50

13 Largo R., Alvarez-Soria M.A., Diez-Ortego I., Calvo E., Sánchez-Pernaute O., Egido J., Herrero-Beaumont G. Glucosamine inhibits IL-1β-induced NFκB activation in human osteoarthritic chondrocytes. *Osteoarthritis Cartilage (2003) 11, 290-298*

14 Hoffer LJ, Kaplan LN, Hamadeh MJ, Grigoriu AC, Baron M.Sulfate could mediate the therapeutic effect of glucosamine sulfate. *Metabolism* 2001;50:767-70.

15 Reginster JY, Deroisy R, Rovati LC et al. Long-term effects of glucosamine sulfate on osteoarthritis progression: a randomized, placebo-controlled clinical trial. *Lancet* 2001;357:251-256

16 Pavelka K, Gatterova J, Olejarova M, Machacek S, Giacovelli G, Rovati LC. Glucosamine sulfate use and delay of progression of knee osteoarthritis: a 3-year, randomized, placebo-controlled, double-blind study. *Arch Intern Med* 2002;162:2113-23

17 McAlindon TE, La Valley MP, Gulin JP, Felson DT. Glucosamine and chondroitin for treatment of osteoarthritis: a systematic quality assessment and meta-analysis. *JAMA* 2000;283:1469-1475

18 Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629-34

19 Sterne JA, Egger M, Smith GD. Systematic reviews in health care: Investigating and dealing with publication and other biases in meta-analysis. *BMJ* 2001;323:101-5.

20 Towheed TE, Anastassiades TP, Shea B, Houpt J, Welch V, Hochberg MC. Glucosamine therapy for treating osteoarthritis (Cochrane Review). In: *The Cochrane Library*, Issue 2, 2001. Oxford: Update Software

21 Lequesne MG, Mery C, Samson M, Gerard P. Indexes of severity for osteoarthritis of the hip and knee. Validation-value in comparison with other assessment tests. *Scand J Rheumatol* Suppl 1987;65:85-89

22 Richy F., Bruyere O., Ethgen O., Cucherat M., Henrotin Y., Reginster J.Y. Structural and symptomatic efficacy of glucosamine and chondroitin in knee osteoarthritis. A comprehensive Meta-analysis. *Arch. Int. Med. 2003; 163: 1514-22*

23 Rindone JP, Hiller D, Collacott E, Nordhaugen N, Arriola G.Randomized, controlled trial of glucosamine for treating osteoarthritis of the knee. *West J Med* 2000;172:91-4

24 Hughes R, Carr A. A randomized, double-blind, placebo-controlled trial of glucosamine sulphate as an analgesic in osteoarthritis of the knee. *Rheumatology* 2002;41:279-84

25 Drovanti A., Bignamini A.A., Rovati A.L. Therapeutic activity of oral glucosamine sulfate in OA: a placebo controlled double-blind investigation. *Clin. Ther* 1980;3:260-72

26 Pujalte JM, Llavore EP, Ylescupidez FR.Double-blind clinical evaluation of oral glucosamine sulphate in the basic treatment of osteoarthrosis. *Curr Med Res Opin* 1980;7:110-14

27 Crolle G, D'Este E.Glucosamine sulphate for the management of arthrosis: a controlled clinical investigation. *Curr Med Res Opin* 1980;7:104-9

28 D'Ambrosio E, Casa B, Bompani R, Scali G, Scali M.Glucosamine sulphate: a controlled clinical investigation in arthrosis. *Pharmatherapeutica* 1981;2:504-8

29 Lopes Vaz A. Double-blind clinical evaluation of the relative efficacy of ibuprofen and glucosamine sulphate in the management of osteoarthrosis of the knee in out-patients. *Curr Med Res Opin* 1982;8:145-9

30 Vajaradul Y. Double-blind clinical evaluation of intra-articular glucosamine in outpatients with gonarthrosis. *Clin Ther* 1981;3:336-43

31 Tapadinhas M.J., Rivera I.C., Bignamini A.A. Oral glucosamine sulphate on the management of arthrosis: report on a multi-centre open investigation in Portugal. *Pharmatherapeutica* 1982;3:157-68

32 Noack W, Fischer M, Förster KK, Rovati LC, Setnikar I. Glucosamine sulfate in osteoarthritis of the knee. *Osteoarthritis Cartilage* 1994;2:51-59

33 Müller-Faβbender H, Bach GL, Haase W, Rovati LC, Setnikar I. Glucosamine sulfate compared to ibuprofen in osteoarthritis of the knee. *Osteoarthritis Cartilage* 1994;2:61-69

34 Qiu GX, Gao SN, Giacovelli G, Rovati L, Setnikar I. Efficacy and safety of glucosamine sulfate versus ibuprofen in patients with knee osteoarthritis. *Arzneimittelforschung* 1998;48:469-74

35 McAlindon T. Glucosamine for osteoarthritis: dawn of a new era? *Lancet* 2001;357:247-248

36 Bruyere O, Honore A, Rovati LC, Giacovelli G, Henrotin YE, Seidel L, Reginster JY. Radiologic features poorly predict clinical outcomes in knee osteoarthritis. *Scand J Rheumatol* 2002;31:13-6

37 Bruyere O, Honore A, Ethgen O, Rovati LC, Giacovelli G, Henrotin YE, Seidel L, Reginster JY. Correlation between radiographic severity of knee osteoarthritis and future disease progression. Results from a 3-year prospective, placebo-controlled study evaluating the effect of glucosamine sulfate. *Osteoarthritis Cartilage* 2003;11:1-5

38 Brandt KD, Mazzuca SA, Conrozier T, Dacre JE, Peterfy CG, Provvedini D, et al. Which is the best radiographic protocol for a clinical trial of a structure modifying drug in patients with knee osteoarthritis? *J Rheumatol* 2002;29:1308-20

39 Pavelka K, Rovati LC, Gatterova J, Bruyere O, Deroisy R, et al. Pain relief is not a confounder in joint space narrowing assessment of full extension knee radiographs. *Osteoarthritis Cartilage* 2002;10(suppl.A):S16-17

40 Reichelt A, Forster KK, Fischer M, Rovati LC, Setnikar I. Efficacy and safety of intramuscular glucosamine sulfate in osteoarthritis of the knee. A randomized, placebo-controlled, double-blind study. *Arzneimittelforschung* 1994;44:75-80

41 Rovati LC. The clinical profile of glucosamine sulfate as a selective symptom modifying drug in osteoarthritis : current data and perspectives. *Osteoarthritis Cartilage* 1997;5(suppl. A): 72

42 Foerster KK, Schmid K, Rovati LC. Efficacy of glucosamine sulfate in osteoarthritis of the lumbar spine: a placebo-controlled, randomized, double-blind study. *Arthritis Rheum* 2000;43(suppl):S335

43 Glucosamine sulfate. Therapy Perspectives 2000. Adis International Ltd, pp:18-20

44 Houpt JB, McMillan R, Wein C, Paget-Dellio SD.Effect of glucosamine hydrochloride in the treatment of pain of osteoarthritis of the knee. *J Rheumatol* 1999;26(11):2423-30

45 http://www.jointandbone.org/news/news071100.b.cfm. Accessed on February 20, 2003

46 Thie NM, Prasad NG, Major PW. Evaluation of glucosamine sulfate compared to ibuprofen for the treatment of temporomandibular joint osteoarthritis: a randomized double blind controlled 3 month clinical trial. *J Rheumatol* 2001;28:1347-55

47 Leffler CT, Philippi AF, Leffler SG, Mosure JC, Kim PD.Glucosamine, chondroitin, and manganese ascorbate for degenerative joint disease of the knee or low back: a randomized, double-blind, placebo-controlled pilot study. *Mil Med* 1999;164:85-91

48 Das A Jr, Hammad TA. Efficacy of a combination of FCHG49 glucosamine hydrochloride, TRH122 low molecular weight sodium chondroitin sulfate and manganese ascorbate in the management of knee osteoarthritis. *Osteoarthritis Cartilage* 2000;8:343-50

49 Conte A, Volpi N, Palmieri L, Bahous I, Ronca G. Biochemical and pharmacokinetic aspects of oral treatment with chondroitin sulfate. *Arzeneimittelforschung* 1995;45:918-25

50 Karzel K, Domenjoz R. Effects of hexosamine derivatives and uronic acid derivatives on glycosaminoglycan metabolism of fibroblast cultures. *Pharmacology* 1971;5:337-45

51 Baron AD, Zhu JS, Zhu JH, Weldon H, Maianu L, Garvey WT. Glucosamine induces insulin resistance in vivo by affecting GLUT 4 translocation in skeletal muscle. Implications for glucose toxicity. *J Clin Invest* 1995;96:2792-801.

52 Monauni T, Zenti MG, Cretti A et al. Effects of glucosamine infusion on insulin secretion and insulin action in humans. *Diabetes* 2000;49:926-35.

53 Pouwels MJ, Jacobs JR, Span PN, Lutterman JA, Smits P, Tack CJ. Short-term glucosamine infusion does not affect insulin sensitivity in humans. *J Clin Endocrinol Metab* 2001;86:2099-2103.

54 Rovati LC, Annefeld M, Giacovelli G, Schmid K, Setnikar I. Glucosamine in osteoarthritis. *Lancet* 1999;354:1640.

55 Scroggie D.A., Albright A., Harris M. The effect of Glucosamine-Chondroitin Supplementation on Glycosylated Hemoglobin Levels in Patients With Type 2 Daibetes Mellitus. A placebo-controlled, Double-blinded, Randomized Clinical Trial. *Arch. Int. Med. 2003; 163: 1587-90*

56 American College of Rheumatology Subcommittee on Osteoarthritis Guidelines. Recommendations for the medical management of osteoarthritis of the hip and knee: 2000 update. *Arthritis Rheum* 2000;43:1905-15.

57 Hochberg MC. What a difference a year makes: reflections on the ACR recommendations for the medical management of osteoarthritis. *Curr Rheumatol Rep.* 2001;3:473-8.

58 Adebowale A.O., Cox D.S, Liang Z., Eddington N.D., Analysis of glucosamine and chondroitin sulfate content in marketed products and the Caco-2 permeability of chondroitin sulfate raw materials. *JAMA 2000; 3: 37-44.*