

**ENGELHARD**

**EquiStat™**

Advanced anti-aging complex



## Product Profile

With a composition of apple and soybean extracts in a non-aqueous butylene glycol vehicle, EquiStat™ presents a unique combination of collagen-boosting, anti-inflammatory and inhibitory compounds. The apple extract contains terpenoid compounds that are proven inhibitors of elastase and possess anti-inflammatory and collagen stimulatory activities. Peptides of the soy hydrolysate in combination with soy isoflavones and the apple triterpenes act synergistically as powerful inhibitors of skin matrix breakdown. This active complex facilitates the development of powerful anti-aging products.

**INCI:** *Butylene Glycol (and) Pyrus Malus (Apple) Fruit Extract (and) Glycine Soja (Soybean) Seed Extract*

**EU INCI:** *Butylene Glycol (and) Pyrus Malus (and) Glycine Soja*

### Summary:

- An active complex with anti-inflammatory, collagen-boosting and matrix metalloprotease inhibitory activities. (*in vitro* study data)
- Main components inhibit collagen breakdown and stimulate collagen synthesis.
- Apple extract helps prevent inflammation, and blocks age- and sun-induced matrix breakdown.
- Soy isoflavones provide anti-aging and wrinkle reduction benefits.

### Essential Components / Technologies:

#### Apple extract:

- Contains the powerful anti-inflammatory agents ursolic acid and quercetin
- Inhibits 5-lipoxygenase and cyclooxygenase, preventing the generation of inflammatory mediators
- Helps prevent the appearance of wrinkles and restores youthful appearance to skin

#### Soy Isoflavones:

- Powerful antioxidants reduce free radical generation
- Inhibit enzymes that mediate connective tissue destruction
- Restore balance between synthesis and destruction of matrix components in aging and post-menopausal skin
- Helps to normalize the rate of epidermal cell proliferation and differentiation seen in aged skin

#### Soy Peptide Complex:

- Provides building blocks for skin protein synthesis
- Contains peptides which are potent metalloprotease inhibitors

*EquiStat™ is a trademark of Engelhard Corporation.*

Engelhard seeks to present reliable information concerning the composition, properties and use of its products, services and processes. However, all literature, advice and other material concerning any product, service or process or its selection or use is provided AT NO CHARGE AND WITH NO WARRANTY OF ANY KIND. All sales are subject to Engelhard's Terms and Conditions of Sale, which are reproduced on the reverse side of each invoice. ALL WARRANTIES OF MERCHANTABILITY AND FITNESS OF PURPOSE ARE DISCLAIMED. Remedies for any breach and Engelhard's liability, including that for patent infringement, are limited as provided in Engelhard's Terms and Conditions of Sale. Engelhard is not liable for consequential, incidental, or special damages. Nothing should be construed as a recommendation or inducement to infringe any patent. No assumption should be made that all safety or environmental protection measures are indicated, or that other measures may not be required.

# Skin Aging Primer

Naturally aged skin is smooth, thin, dry, pale and finely wrinkled. In contrast, photoaged skin is dry, thick and coarsely wrinkled. A variety of changes occur both with age and sun exposure. These changes include:

- **Structural changes** in the content and architecture of skin collagen
- **Functional changes** such as changes in skin antioxidant defense systems and hormone levels
- **Changes in skin responses** such as inflammatory responses and wound healing responses.

## *Collagen and skin texture:*

Collagen is a natural protein that provides structural support for connective tissues and gives skin its firmness and tensile strength. It is found throughout the body — in skin, muscle, tendon, and bone. In the skin, collagen provides texture, resiliency, elasticity and shape. Collagen and elastin are produced in the dermis by fibroblasts, the major cells of the dermis. Collagen is the most abundant protein of the skin, accounting for 70% of the total protein content. The fibroblasts also produce elastin that contributes to skin elasticity. Fibers of collagen, elastin and other proteins are woven together like threads in fabric to form a framework called extracellular matrix (ECM), into which new cells can grow. Collagen and elastin contribute structure, strength, elasticity and durability.

## *Changes with age and photoage:*

Collagen breakdown increases with chronological aging and photoaging<sup>1</sup>. Sun damage, free radicals, age-related hormonal changes, and smoking are all factors that contribute to this breakdown. Fibroblasts normally replace damaged collagen fibers with new ones. However, the ability of fibroblasts to replace collagen is also compromised by natural aging and environmental stress.

Increased collagen breakdown leads to thinning and loss of the elastic fiber network in the dermis. This results in the formation of wrinkles. Sun-exposed areas of the skin are most prone to wrinkles and imperfections.

## *Menopause and skin aging:*

Significant changes happen to skin during menopause<sup>2</sup>. These changes include:

- Diminished skin thickness
- Decreased proliferation and differentiation of skin cells
- Reduced production of collagen and other cellular matrix components
- Increased activity of proteases that degrade matrix components
- Diminished wound healing ability and increased wrinkles and dryness.

Hormone replacement therapy (HRT) or topically applied estrogen can help to prevent or check most of the changes associated with postmenopausal skin<sup>3</sup>. A natural alternative to HRT, soy isoflavones, may be effective in mitigating these changes as well.

### Matrix Metalloproteases (MMPs):

The collagen content of skin is the net balance between collagen synthesis and collagen breakdown mediated by matrix metalloproteases (MMPs). Age reduces collagen synthesis in human tissues including the skin. Environmental stress such as smoking, UV exposure, pollution and inflammation stimulate MMPs, further accelerating collagen breakdown. All these processes upset the matrix equilibrium within human skin. Tissue Metalloprotease Inhibitors (TIMPs) are endogenous inhibitors of MMPs. The activity and the production of MMPs and TIMPs are regulated by various protein factors called cytokines.

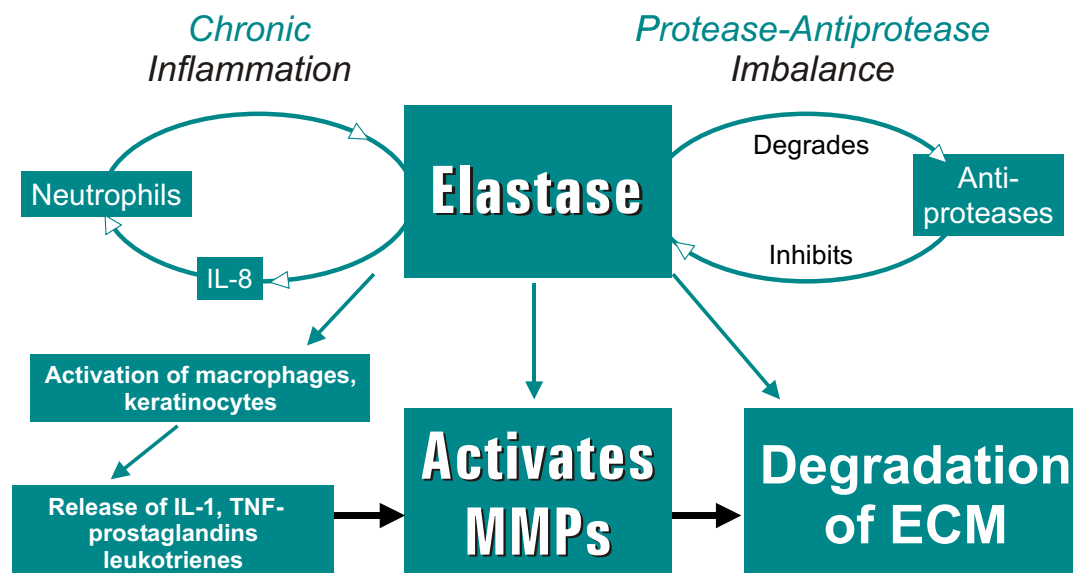
Anti-inflammatories can prevent MMP activation (genomic approach) and ultimately help to reduce collagen breakdown. Direct inhibition of MMPs by plant compounds (proteomic approach) is another method to mitigate collagen breakdown in the skin.

### Free radicals and skin aging:

Environmental stresses and the natural aging process generate free radicals and reactive oxygen species. This in turn stimulates the skin's inflammatory response. The skin of both aged and photoaged individuals has been shown to have reduced levels of the natural enzymatic and non-enzymatic antioxidant defense mechanisms, thereby making the skin more vulnerable to infection and inflammation<sup>4</sup>.

### Inflammation activates MMPs:

Aging and photoaging cause neutrophils (an infection-fighting cell type in the blood) to infiltrate skin and release an enzyme called elastase. Normally, antioxidant defense mechanisms prevent neutrophil infiltration into skin. In normal skin, a balance exists between the levels of elastase and elastase inhibitors. When this equilibrium is disturbed, elastase breaks down elastins as well as initiating events that result in inflammation. Neutrophils attract another cell type from the blood, macrophages. They release pro-inflammatory cytokines, such as TNF-alpha, and interleukin-1 and 8, which stimulate additional neutrophil influx. Inflammation also causes cytokine release from skin cells such as keratinocytes. These cytokines cause a cascade of reactions that lead to increased release of elastase. Activation of 5-lipoxygenase and cyclooxygenase cause increased production and release of inflammatory mediators such as prostaglandins and leukotrienes. All these events result in MMP synthesis (gene activation or genomic process) and/or the stimulation of enzymatic activity of MMPs (proteomic process).



## How Does EquiStat Work?

*The natural components of EquiStat prevent inflammation, promote collagen synthesis, and inhibit skin matrix degradation:*

### *Apple Extract:*

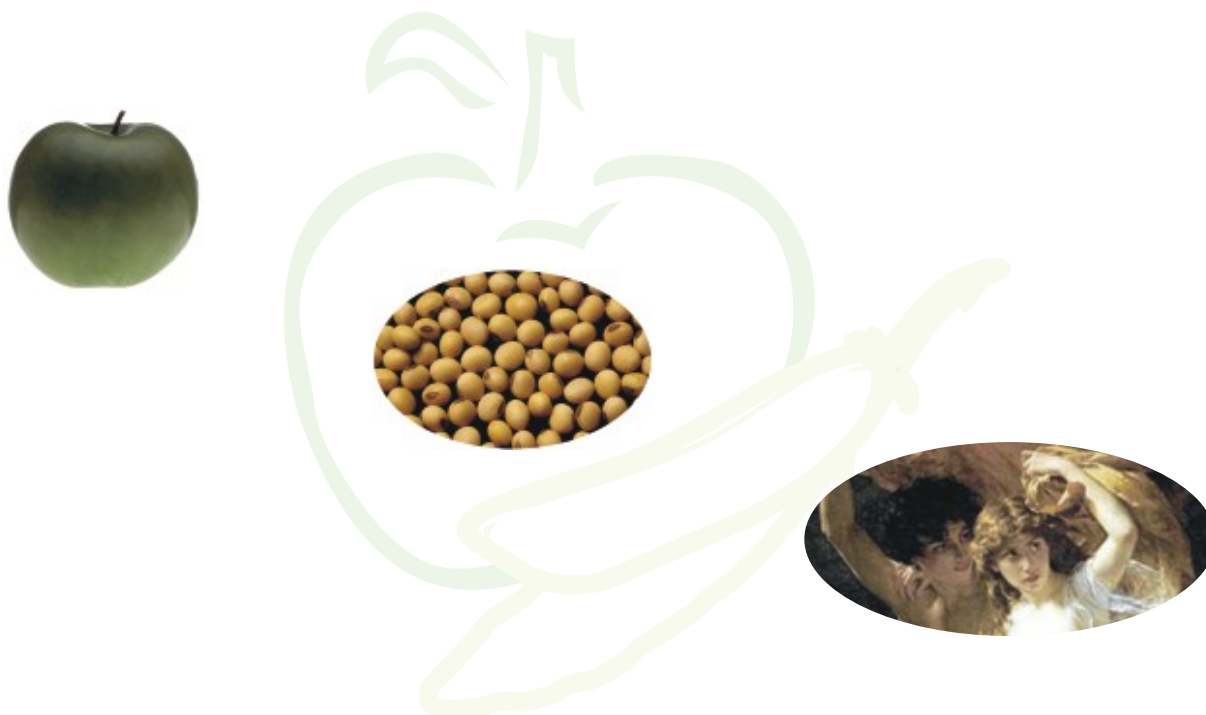
Terpenoids (such as ursolic acid, present in apple extract) inhibit human leukocyte elastase, 5-lipoxygenase and cyclooxygenase activities<sup>5,6</sup>. These effects prevent inflammation by multiple mechanisms. Ursolic acid also stimulates skin collagen synthesis and epidermal cell ceramide synthesis<sup>7</sup>. Ursolic acid has been used successfully to treat photoaged skin. Ursolic acid helps to reduce the appearance of wrinkles and age spots by restoring the skin's collagen structure and elasticity<sup>8</sup>.

### *Soy Isoflavones:*

The major isoflavone components of soy (genistein, daidzein and their glycosides) are powerful antioxidants and prevent reactive oxygen species generation, providing anti-inflammatory benefits with a similar end result but different mechanism than the apple extract<sup>9</sup>. This helps to control the amplified MMP activity normally seen in post-menopausal women. In addition, isoflavones stimulate collagen synthesis and prevent the skin thinning that occurs with menopause<sup>10</sup>. Genistein and daidzein stimulate hyaluronic acid production in skin cells in culture and *in vivo*<sup>11</sup>. Thus, the net effect of soy isoflavones in menopausal skin is to prevent inflammation, stimulate collagen synthesis and inhibit collagen breakdown.

### *Soy Peptide Complex:*

The soy peptide complex used in EquiStat has been specially formulated to provide maximal MMP inhibitory activity. The hydrophobic di-peptides present in the soy peptide complex bind and inactivate the MMP active site and prevent MMP from degrading collagen bundles. They provide essential amino acids for the synthesis of skin collagen and interact synergistically with the other components to improve the skin condition and provide anti-aging benefits.



# Performance Evaluation

## *In vitro* Matrix Metalloproteinase (MMP) Inhibition Assay

Matrix metalloproteinase inhibitors (MMPi) from different commercial sources were tested for their ability to inhibit MMPs using a fluorogenic peptide assay system. In this system, the substrate is a fluorogenic peptide which undergoes an increase in fluorescence when cleaved by the MMP. The reaction is followed by monitoring the increase in the fluorescence of the reaction mixture for 2 hours. The rate of fluorescence increase is a measure of the reaction rate.

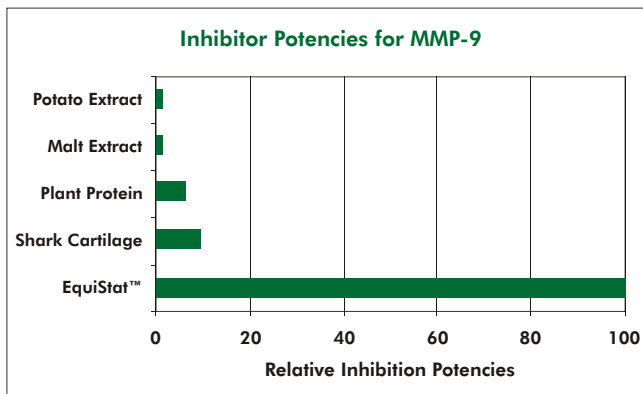
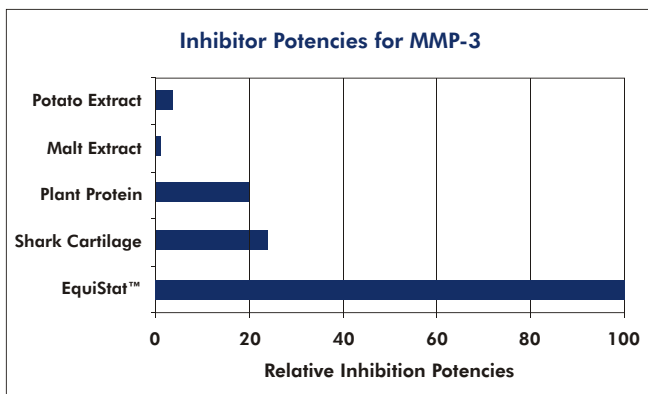
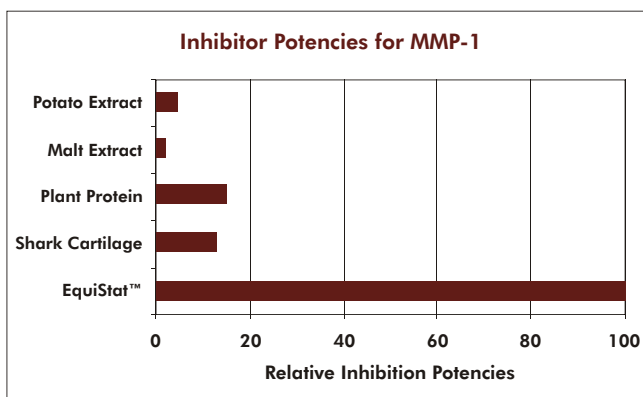
The assay is performed in microtiter plates using triplicate wells per condition. The test sample contains specific MMP and the putative MMP inhibitor at several concentrations and the mean rate of the reaction at each of these conditions is compared to the reaction rate in the absence of inhibitor.

The concentration of the test sample that inhibits the reaction rate (i.e., MMP inhibition) by 50% is defined as the "50% Inhibitory Concentration" or  $IC_{50}$ . This is the most commonly employed parameter indicating enzyme inhibition. A potent inhibitor has a low  $IC_{50}$  value. The  $IC_{50}$  values are indicated in the table below.

For ease of comparison, relative "inhibitor potencies" of the different inhibitors are shown in comparison to EquiStat normalized to 100%. This was calculated by using the inverse of the  $IC_{50}$  values to obtain the "potency" for each of the inhibitors and then expressing this as a percentage relative to EquiStat, set to 100%. For example, a material with a relative inhibition potency of 20% would be 1/5 as potent as EquiStat.

**50% Inhibitory Concentration ( $IC_{50}$ )**

	MMP-1	MMP-3	MMP-9
Potato Extract	15.00	15.00	20.00
Malt Extract	35.00	40.00	20.00
Plant Protein	4.40	3.00	4.40
Shark Cartilage	5.00	2.52	3.00
EquiStat™	0.65	0.60	0.28



# Performance Evaluation

## *In Vivo Efficacy of EquiStat:*

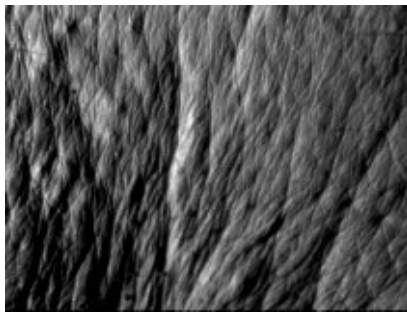
The efficacy of EquiStat in reducing the visible signs of aging at the crow's foot area of the face is shown in the study described below:

### *Methodology:*

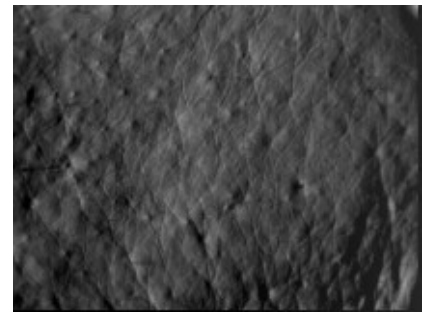
Twenty Subjects (35-65 y.o.) having mild to moderate fine lines & wrinkles at the crow's foot area of the face were recruited for the study. Subjects were provided with a cleansing soap for their facial cleansing. Twice a day, following each wash, subjects applied test products to their crow's foot area of the face. Subjects were evaluated for the reduction in the number and depth of wrinkles and reduction in the roughness after four (4) weeks of product use. Changes from baseline were calculated for each parameter. Ten subjects applied base only; ten subjects applied base containing 1.25% EquiStat.

### *Results:*

Silicone replicas of the baseline and 1.25% EquiStat treated skin for 4 weeks. After 4 weeks of product application, the skin looks smoother, and the wrinkle numbers and size were diminished.



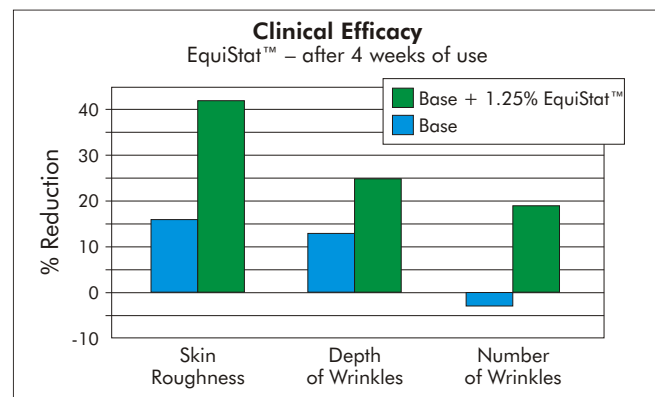
**Baseline**



**After 4-week 1.25% treatment**

90% of the subjects (9 of 10) showed a reduction in the wrinkle number and depth of wrinkles. All the subjects showed reduction in the roughness of skin after the product application.

Quantitation of the number depth and roughness of wrinkles is shown in the adjacent figure.



## **Summary**

- Four weeks of treatment with 1.25% EquiStat reduced the number of wrinkles significantly by 19%.
- The average depth of the wrinkles decreased in control by 12%, but the depth of wrinkles decreased by 25% after 4 weeks of treatment with EquiStat.
- Four weeks treatment with EquiStat reduced the skin roughness by 45% (vs. 16% for controls).

In conclusion, four weeks of treatment with 1.25% EquiStat reduced the number and depth of wrinkles, and reduced the skin roughness in 90% of the subjects.

# Product Details

## Usage/Application

Usage Levels: 1-5% in a final product

Application: EquiStat can be used in anti-aging, anti-wrinkle and moisturizing skin care products for all types of skin (dry, normal, and sensitive). It can be used in post-sun products and is particularly useful in products intended for the treatment of mature skin. It may also be used in treatment makeup products.

## Compatibility/ Formulation Tips

No compatibility issues found when used with conventional ingredients. Consider the following in regard to EquiStat:

- It may be used within a pH range of 4.5-7.5.
- It must be incorporated at temperatures lower than 50° C.
- Depending upon the formulation, it can be added by itself, dissolved in a solvent such as a glycol, or blended with "claim" ingredients as long as no incompatibility is present.
- If water is to be added to the sub-phase containing EquiStat, the time for addition of the sub-phase including water to the finished formulation must be minimized.
- The concentration of the product to be added to the final formulation may be "dialed" to provide mild to intense anti-aging effects to suit the development profile.
- Make sure the vessel containing EquiStat to be added to the formulation is kept well covered.

## Scientific Designations

Product: EquiStat™, R10714			
INCI	Butylene Glycol	Pyrus Malus (Apple) Fruit Extract	Glycine Soja (Soybean) Seed Extract
CAS#	107-88-0	85251-63-4	-
EINECS#	203-529-7	286-475-7	-
Japan Regis. #	100040 (1,3-Butylene Glycol)	100606 (Apple Extract)	520734 (Soy Extract)

## Solubility

Solvent	Solubility
Deionized water	Soluble
100% ethanol	Soluble
25:75 alcohol:water	Soluble
50:50 alcohol:water	Soluble
75:25 alcohol:water	Soluble
Corn oil	Suspension
Olive oil	Suspension
Mineral oil	Insoluble
Butylene glycol	Soluble
Propylene glycol	Soluble
Silicone oil	Insoluble
Isononyl isononanoate	Soluble
SLES surfactant	Suspension

## Safety

In an ocular irritation assay, EquiStat displayed minimal irritation at use levels of 2–5%.

HRIPT: At normal use levels, EquiStat does NOT indicate a potential for dermal irritation or a potential for allergic contact sensitization.

## Typical Properties

Specification	Typical Values
Color	Pale Straw
Odor	Characteristic
Appearance	Hazy to Transparent Liquid (Precipitation May Occur)
Microbiology	Bacteria & Fungi: <100 cfu/g Pathogens: Free
Storage	Keep sealed at room temperature in a dry place. Store between 20 – 25°C.

## References:

1. Voorhees JJ et al., J. Investig. Dermatology., 114: 480, 2000.
2. Raine-Fenning NJ, Brincat MP, Muscat-baron Y., Am. J. Clin. Dermatol., 2003, 4: 371.
3. Dunn LB et al., Arch Dermatol., 133: 339, 1997.
4. Gieun Rhie et al., J. Investig. Dermatology., 117: 1212, 2001.
5. Ying QL, Rinehart AR, Simon SR Bochem J. 1991 Jul 15;277 ( Pt 2):521-6.
6. Najid, A., Simon, A., Cook, J., Chable-Rabinovitch, H., Delage, C., Chulia, A., and Riguad, M. (1992). FEBS 299(3), 213-217.
7. Arch Dermatol Res. 2002 Jan;293(11):569-75.
8. Liu, J. (1995) Pharmacology of oleanolic and ursolic acid. J. of Ethnopharmacology 49, 57-68.
9. Wei H, Bowen R, Cai Q, Barnes S, Wang Y. Proc Soc Exp Biol Med. 1995 Jan;208(1):124-30.
10. Kritiz-Silverstein D, Goodman-Gruen DL.. J Womens Health Gend Based Med. 2002 Jan-Feb;11(1):69-78.
11. Miyazaki K, Hanamizu T, Iizuka R, Chiba K.,, Skin Pharmacol. Appl. Skin Physiol., 2002, 15: 175-83.

**ENGELHARD**

Change the nature of things.

[www.engelhard.com/pcm](http://www.engelhard.com/pcm)

**Engelhard Corporation**

50 Health Sciences Drive  
Stony Brook, NY 11790 USA

Telephone: (631) 689-0200

Fax: (631) 380-2550

e-mail: [pcmsales@engelhard.com](mailto:pcmsales@engelhard.com)