

ActiGuard™ S

NATURAL PERFORMANCE INGREDIENT



CTFA/INCI Name:

Sorghum Vulgare Seed/Skin/Stalk Extract
Glycerin
Water

CAS Numbers:

97676-10-3, 56-81-5, 7732-18-5

EINECS/ELINCS Number:

307-686-3, 200-289-5, 231-791-2



WHAT IS ACTIGUARD™ S NPI?

ActiGuard™ S Natural Performance Ingredient (NPI) is the first in Active Organics® newest line of botanical actives, designed around extracts of the ancient grain Sorghum. ActiGuard™ S performance ingredient is a glycerin and water extract of Sorghum Vulgare, which is rich in polyphenolic phytochemicals. The flavonoids, condensed tannins, and phenolic compounds extracted from the bran portion of this ancient grain result in substantial anti-inflammatory and anti-irritation properties.

WHAT DOES IT DO?

ActiGuard™ S performance ingredient helps protect the skin by helping to reduce the immediate activity of the inflammatory response. *In vivo* tests show ActiGuard™ S performance ingredient helps reduce skin neurosensory irritation and red blood cell concentration due to skin inflammation. *In vitro* tests have shown ActiGuard™ S performance ingredient helps attenuate inflammation of the skin through inhibition of PAR-2 evoked IL-8 release.

WHAT IS IT FOR?

ActiGuard™ S Natural Performance Ingredient is ideal for use in green and sustainable product lines, where a high level of bio-activity is required. Perfect for use in sensitive skin products, ActiGuard™ S performance ingredient is designed to help minimize inflammatory skin conditions.

HOW IS IT DIFFERENT?

Due to Active Organics® specialized, proprietary extraction process, ActiGuard™ S Natural Performance Ingredient is rich in bio-active phytochemicals and gets all of its bio-activity from a completely natural source, Sorghum bran. Shown to be a very effective agent in helping reduce skin irritation, Sorghum itself contains phytochemicals and polyphenolic compounds with a wide range of published biological activities. ActiGuard™ S performance ingredient has been able to enhance these properties to take advantage of one of the best ingredients that nature has to offer:

HOW DO I USE IT?

ActiGuard™ S performance ingredient is designed to be used from 3-10% in daily use products such as creams, lotions, gels, and serums. ActiGuard™ S performance ingredient should be used in after sun products, sunscreens, exfoliants, calming products for sensitive skin, cleansing products, and anti-acne treatments. Ideal for sensitive, inflamed and overly reactive skin conditions, ActiGuard™ S Natural Performance Ingredient is water soluble and should be added to the final stage of production at 45°C or less.



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SENSITIVE SKIN & ACTIGUARD™ S

INTRODUCTION

ActiGuard™ S Natural Performance Ingredient is the first product in Active Organics® newest line of botanical actives, designed around bran extracts of the ancient grain, Sorghum Vulgare — rich in Polyphenolic phytochemicals, flavonoids, and condensed tannins. **ActiGuard™ S** performance ingredient is ideal for use in green and sustainable products, where a high level of bio-activity is required.

Sorghum is among the five most important cereal crops in the world. As a true ancient grain, its oldest cultivation records date back to 3000 B.C. in Egypt. It is a drought-tolerant crop with special adaptations to weather extremes. It is among the most efficient crops in conversion of solar energy and use of water, making it a green, renewable crop with a lower carbon footprint than corn.¹

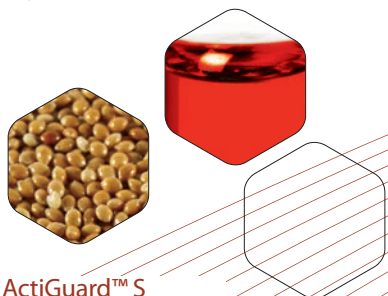
Mother Nature is an outstanding chemist and has created a diversity of plants and foods with a wide range of biochemical components. Sorghum bran is known to have a high content of antioxidants, including phenolic acids, flavonoids, and condensed tannins.² Due to our specialized, proprietary extraction process of Sorghum bran, **ActiGuard™ S** performance ingredient is rich with these bio-active phytochemicals and can help reduce skin irritation by helping mediate the inflammatory response induced via skin irritants and oxidants. This **ActiGuard™ S** performance ingredient has shown *in vivo* the ability to reduce neurosensory irritation and mediate the inflammatory response via reduction in red blood cell concentration. **ActiGuard™ S** performance ingredient has also demonstrated *in vitro* anti-inflammatory effects by inhibiting the levels of pro-inflammatory cytokine IL-8 released by primary human keratinocytes upon PAR-2 activation.

Sensitive skin is not a medical term but can be defined from a consumer perspective as an atypical reaction to external stimuli including the application of topical products. Sensitivity usually arises from one of three different causes including:

- Fragile skin which has an impaired barrier function
- Inflamed skin either from an acute trauma or a chronic clinical or sub-clinical skin condition
- Overly reactive skin due to a low threshold of neural stimulation

Regardless of the cause, sensitive skin usually has similar characteristics including a stinging, burning, itching, erythemic response and/or pain to stimuli including, heat, electrical stimulation or application of chemicals. At Active Organics®, we have designed **ActiGuard™ S Natural Performance Ingredient** to help reduce both the neurosensory irritation and the inflammation caused by the application of topical products.

Inflammation is known as one of the most severe instigators of skin damage and unfortunately, sensitive skin can make inflammation worse. Inflammation is noted by an increased number of red and white blood cells that rush to the site of skin damage to help the skin recover. Inflammation destroys healthy skin cells by triggering free radicals. Because free radicals steal electrons from other molecules, rendering those molecules damaged, they both trigger inflammation and are created by it.³ It is important to protect the skin by mediating irritation and inflammation to help soothe and calm skin. With its anti-irritation and anti-inflammatory effects, **ActiGuard™ S** performance ingredient is perfect for use in all types of anti-irritation products and is especially valuable when designing products for sensitive, inflamed, or overly reactive skin.



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Today botanical extracts have an important role in cosmetic product development; namely bio-functionality and specific skin benefits. *In vivo* and *in vitro* screening studies have correlated well with published literature concerning the bio-functionality of **ActiGuard™ S** performance ingredient. With the wide range of biological activities stemming from a natural, eco-friendly source, **ActiGuard™ S** performance ingredient is perfect for use in sensitive skin and anti-irritation formulations.

Summary of Testing Results:



- Neurosensory Irritation (NSI) induced by Lactic Acid was reduced by 39% after treatment with 3% **ActiGuard™ S Natural Performance Ingredient**.
- Skin irritation as measured by red blood cell concentration during an inflammatory reaction was reduced by as much as 21% with 10% **ActiGuard™ S Natural Performance Ingredient**.
- Levels of pro-inflammatory cytokine IL-8 released by primary human keratinocytes upon PAR-2 activation were inhibited up to 17% with 0.5% **ActiGuard™ S Natural Performance Ingredient**.

ACTIGUARD™ S IN-VIVO TESTING RESULTS

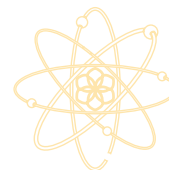
Neurosensory Irritation (NSI) Assay

Assessment of skin irritation can be done by many means including a clinical grading, a measurement of skin color with the Minolta Meter, or assessment of skin blood flow or red blood cell concentration. The first method we have found useful is to measure skin sting response or more commonly referred to as Neurosensory Irritation (NSI). A stinging, burning or itch response can be induced by numerous stimuli, including capsaicin, alcohol, hydroxy acids, nicotinate or derivatives, and temperature or pH changes. Generally these topical changes result in activation of the Transient Receptor Potential Vanilloid Receptor Proteins (TRPV1) on skin nerve cells and this initiates a cascade of reactions resulting in a reaction ranging from a simple sting to a full blown erythemic reaction. For this test, 10 pre-screened subjects were selected without having applied any product to their facial area for at least 48 hours prior to the test. The morning of the test, subjects washed with a popular name brand soap and then cleaned the nasal fold area with 95% alcohol. Subjects were equilibrated for at least 30 minutes in a temperature controlled room.

Testing procedure: a 3% **ActiGuard™ S** performance ingredient solution is applied to one side of the nasal fold area, and as a control water is applied to the other side. After 10 minutes, a 12% lactic acid test solution is applied to the nasal fold area on each side of the face.

Subjects rate skin sting/irritation reaction (NSI) on a 0-4 scale as follows:

- 0 no irritation whatsoever
- 1 slight sting, barely noticeable
- 2 constant noticeable sting
- 3 marked sting, distracting, some burning
- 4 distracting stinging, somewhat painful, burning itching



Evaluations were made at 0, 1, 2, 3, 4, 5, 7, 10, 12 and 15 minutes after application of the test material. After 15 minutes a total NSI score is compared by summing up the individual scores at each time point for the control and treatment sites.

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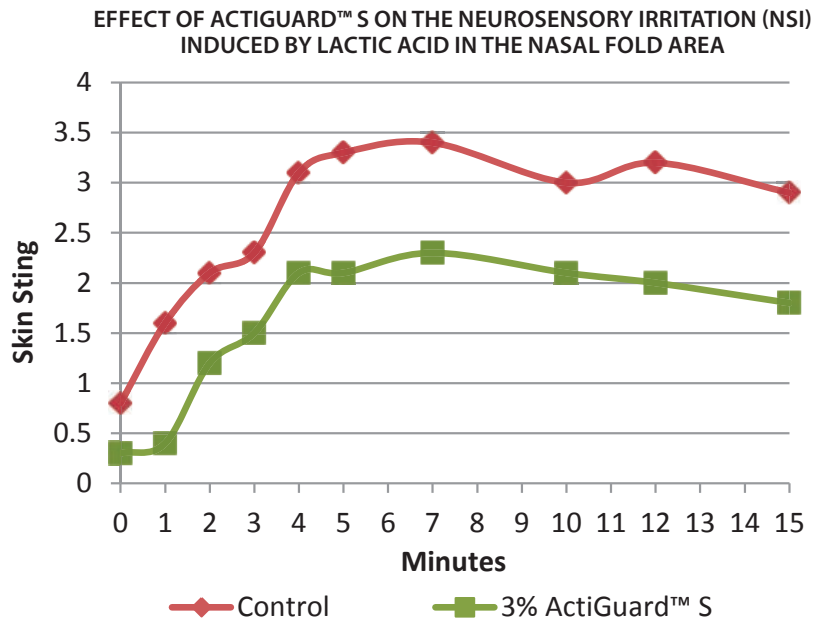
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ACTIGUARD™ S IN-VIVO TESTING RESULTS

Neurosensory Irritation (NSI) Assay (continued)

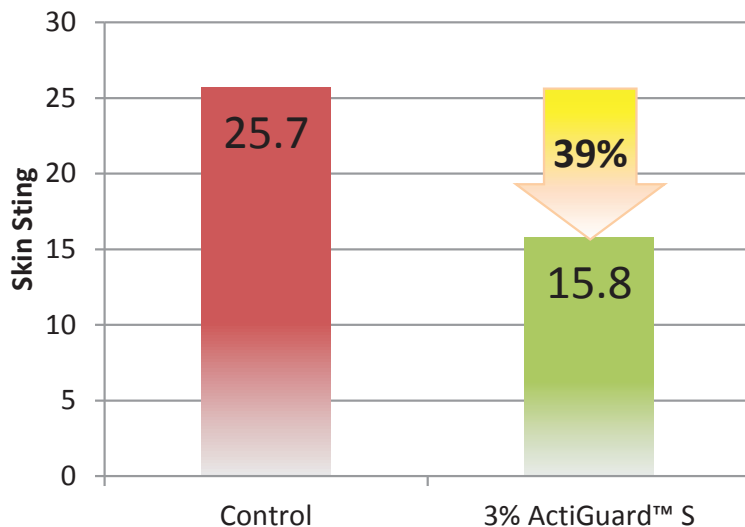
The average of each test subjects score is shown in Table 1.

TABLE 1



As shown in Table 2, 3% ActiGuard™ S performance ingredient in water reduced skin Neurosensory Irritation (NSI) induced by Lactic acid treatment in the nasal fold area by 39% compared to a control (water only). ActiGuard™ S Natural Performance Ingredient as tested is very effective at reducing the sting response in skin.

TABLE 2



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ACTIGUARD™ S IN-VIVO TESTING RESULTS



Inflammatory Response by Sub-epidermal Imaging

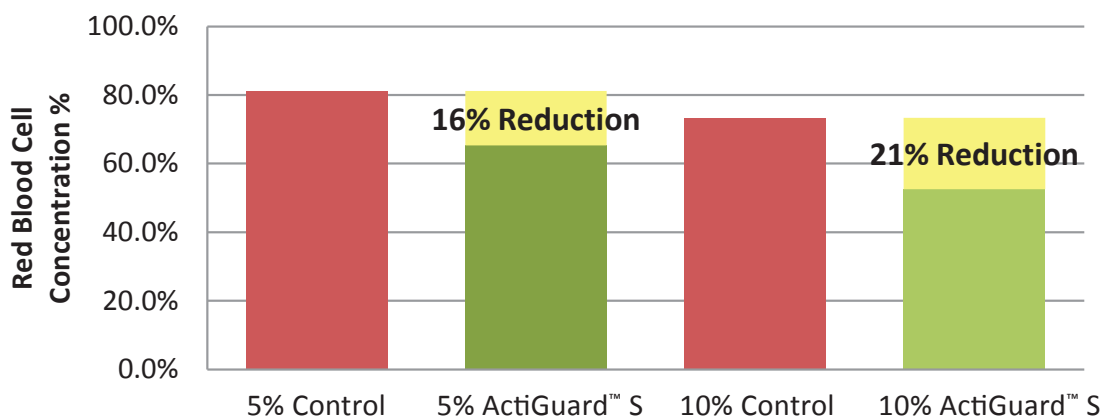
Another method we used to evaluate an inflammatory reaction is to measure red blood cell concentration under the skin using Tissue Viability Imaging (Tivi) technology that combines polarization spectroscopy with advanced image processing. The imager can “see through” the top layer of the skin and collect information about the underlying microcirculation and other skin parameters.⁴ Linearly polarized white light is partly reflected by the upper layer of the skin and partly diffusely scattered in the deeper dermal layers where the microvascular network is located. Most of the directly reflected light preserves its state of linear polarization, while the light diffusely scattered in the tissue successively becomes randomly polarized. The backscattered linearly polarized light directed towards the detector is effectively blocked by a filter with a polarization direction perpendicular to that of the linearly polarized light. A portion of the randomly polarized light passes through this filter and reaches the detector. This arrangement gives the impression that the camera can see through the top layer of the skin and probe the microcirculation in the deeper dermal layers.⁵ By recording the concentration of red blood cells that pass through the tissue, we can quantitate the inflammatory response.

Testing procedure: Polarized digital time lapse photos were taken of the volar forearm of healthy test subjects, with no history of skin disease, and who had not used topical or systemic corticosteroid preparations in the previous two months. All subjects were nonsmokers. Subjects arrived in the test center with no product applied to the volar forearm and cleansed their arm with an alcohol wipe 15 minutes prior to the test. The subjects were acclimatized in the test center at 21°C for 30 minutes and rested in a seated position with the volar forearm placed on a flat table at heart level. The instrument was positioned above the forearm for data acquisition. Each subject's forearm is pretreated with 5% or 10% **ActiGuard™ S Natural Performance Ingredient** on one side and a control (water) on the other. After 15 minutes, a 0.1% Methyl Nicotinate solution is applied to both sides of the forearm, and photos are taken every 10 seconds for a 10 minute period.

Table 3 shows the average reduction in red blood cell concentration over the period of irritation. As you can see, pretreatment with 5% **ActiGuard™ S** performance ingredient reduced skin irritation by 16% and 10% **ActiGuard™ S** performance ingredient reduced skin irritation by 21%.

TABLE 3

EFFECT OF ACTIGUARD™ S ON THE RED BLOOD CELL CONCENTRATION INDUCED BY METHYL NICOTINATE ON THE VOLAR FOREARM



*Average of three measurements at each concentration is calculated. Baseline (non-treated) measurements are subtracted from Control and **ActiGuard™ S Natural Performance Ingredient** test areas for each reading to ensure no background interference.

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ACTIGUARD™ S IN-VITRO TESTING RESULTS

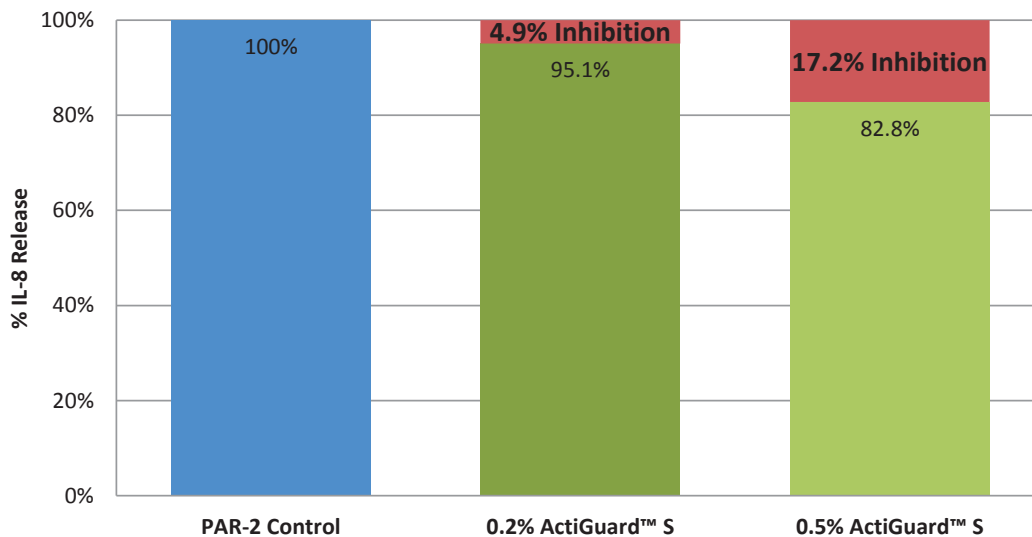
Inhibition of PAR-2 Evoked IL-8 Release (Skin Inflammation)

Proteinase-Activated Receptor-2 (PAR-2) is a protein coupled receptor that modulates the inflammatory response. PAR-2 activation amplifies neurogenic and acute inflammation via the release of pro-inflammatory cytokines. Thus a way to attenuate inflammation is to down-regulate PAR-2 activity to reduce the levels of pro-inflammatory cytokines. The specific aim of this study was to evaluate the efficacy of **ActiGuard™ S Natural Performance Ingredient** on the inhibition of the release of the pro-inflammatory cytokine IL-8 induced by PAR-2 activity in culture supernatants from primary human keratinocytes by ELISA. Cell cultures were incubated with **ActiGuard™ S** performance ingredient in the presence of PAR-2 agonist (activator) for 48 hours at 37°C in a CO₂ incubator. Culture supernatants were harvested and the amount of cytokine IL-8 released determined immediately after collection by an ELISA test. ELISA results were normalized with the total cell number calculated by the Crystal Violet DNA staining assay and quantified by absorbance at 630nm.

As you can see in Table 4, **ActiGuard™ S Natural Performance Ingredient** was effective at inhibiting the levels of pro-inflammatory cytokine IL-8 released by primary human keratinocytes upon PAR-2 activation up to 17%. This shows that **ActiGuard™ S** performance ingredient has statistically significant *in vitro* results to help inflammatory skin conditions.

TABLE 4

EFFECT OF ACTIGUARD™ S ON THE INHIBITION OF THE RELEASE OF THE PRO-INFLAMMATORY CYTOKINE IL-8



ActiGuard™ S Natural Performance Ingredient was effective up to 17% at inhibiting the levels of pro-inflammatory cytokine IL-8 released by primary human keratinocytes upon PAR-2 activation.

DISCUSSION

ActiGuard™ S Natural Performance Ingredient has shown strong anti-inflammatory and anti-irritation effects. In reducing neurosensory irritation and reducing red blood cell concentration, **ActiGuard™ S** performance ingredient reduces the likelihood of skin irritation due to the inflammatory response. As a bio-functional cosmetic additive, **ActiGuard™ S Natural Performance Ingredient** is useful for all types of products designed to provide relief for sensitive, inflamed, and overly reactive skin conditions.

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Notable Publications

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The information presented applies to cosmetic product use and is intended for information only.
It is the responsibility of the user of this ingredient to test finished product for safety and claim substantiation purposes.

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