

# A Comprehensive View of Surfactant Irritation Potential

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## Overview

As modern consumers continue to seek new and improved ways to optimize their cleansing routines, one trend stands out over others: the need for more gentle cleansing. And as animal testing for irritation has been replaced by a wide array of in-vitro tests, each with its own result scale and inherent challenges, it has become difficult to compare results across a broad array of products. Vegan-compliant lab scale zein testing has been conducted on over 60 individual surfactant ingredients and commercial cleansing products, covering traditional chemistry as well as new innovations in all major surfactant types: anionic, cationic, amphoteric, and nonionic. The same method is used to benchmark and evaluate formulation modifications with the goal of reducing the potential for irritation in vivo.

## 1. Introduction

Consumer safety is the most vital component of sustainable product development. Until recently, predicting skin and eye irritation in consumers was done primarily with animal models. Recognizing the potential for suffering in mammals, efforts have been made to develop humane animal-free models with good prediction power. Today, a large variety of test protocols are available, which has achieved the goal of reducing animal suffering. Unfortunately, it has also created challenges in comparing products.

Various globally recognized OECD methods all have specific nuances in how they react to irritants, from the closest animal analog, the Bovine Corneal Opacity and Permeability Assay (BCOP) to Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) to propriety cell models like MatTek EpiDerm™ and EpiOcular™ to in chemico models like InVitro International's Irritector® and now *in silico* "AI" models.

Zein, a corn protein that is similar to keratin present in the skin and hair, is insoluble in water unless it is denatured. Protein denaturation is considered to be a good predictor of skin and eye irritation *in vivo*<sup>1</sup> and the use of zein as a proxy for human proteins and irritation potential as been well established<sup>2,3</sup>, demonstrating acceptable correlation, though generally insufficient to achieve global acceptance for regulatory bodies. The goal of this study is to develop a single, large body of zein data using a modified zein method that offers good repeatability with almost no specialized equipment necessary.

## 2. Materials

Surfactants and surfactant mixtures, with the exceptions of those noted were supplied by Colonial Chemical, Inc, South Pittsburg, TN. Zein protein was purchased from Spectrum Chemical, New Brunswick, NJ. Whatman filter papers were purchased from VWR, Radnor, PA. Additional surfactants and mixtures are Genamin® CTAC 50 (Cetrimonium Chloride), Plantapon® ACG HC (Sodium Cocoyl Glutamate), Pureact I-78 C (Sodium Cocoyl Isethionate), Iselux® LQ-CLR (Sodium Lauroyl Methyl Isethionate), Hamposyl™ L30K (Sodium Lauroyl Sarcosinate), and Pureact WS Conc (Sodium Methyl Cocoyl Taurate).

## 3. Methodology

Surfactant test solutions were prepared at concentrations of 3.0% solids in deionized water. Recorded weights of zein powder were added to 50 mL of the test solutions and mixed for 60 minutes. The solutions were then poured onto filter papers (Whatman 3, 90 mm) of known weights seated inside a ceramic Büchner funnel under vacuum. After vacuum filtration, the filter papers and collected zein were dried in a convection oven at 110°C for 60 minutes. The weights of the collected, oven-dried zein were used to determine the amounts of denatured and dissolved zein in the following formula, where Z represents the initial weight of zein, IW represents the initial weight of the filter paper, and FW represents the final combined weight of the filter paper and collected zein. All results were measured in triplicate.

$$\text{Zein Dissolved (mg/g solid product)} = \frac{Z - (FW - IW)}{1.5} \times 1000$$

The amount of zein that has been denatured and dissolved is presented as "mg zein dissolved /g solid surfactant," where a lower number indicates lower potential for irritancy. This differs from the traditional "zein score", which is based on N analysis. Normalizing all data to solids allows for more ready comparisons of commercial products, where determination of true actives may be difficult.



Protein after mixing and drying using one surfactant with a high degree of denaturation/anticipated irritation (Left) and one with a low degree of denaturation (Right)



## Results

## Irritation Potential by Zein Test

Avoid irritation using the mildest plant-based products from Colonial Chemical.

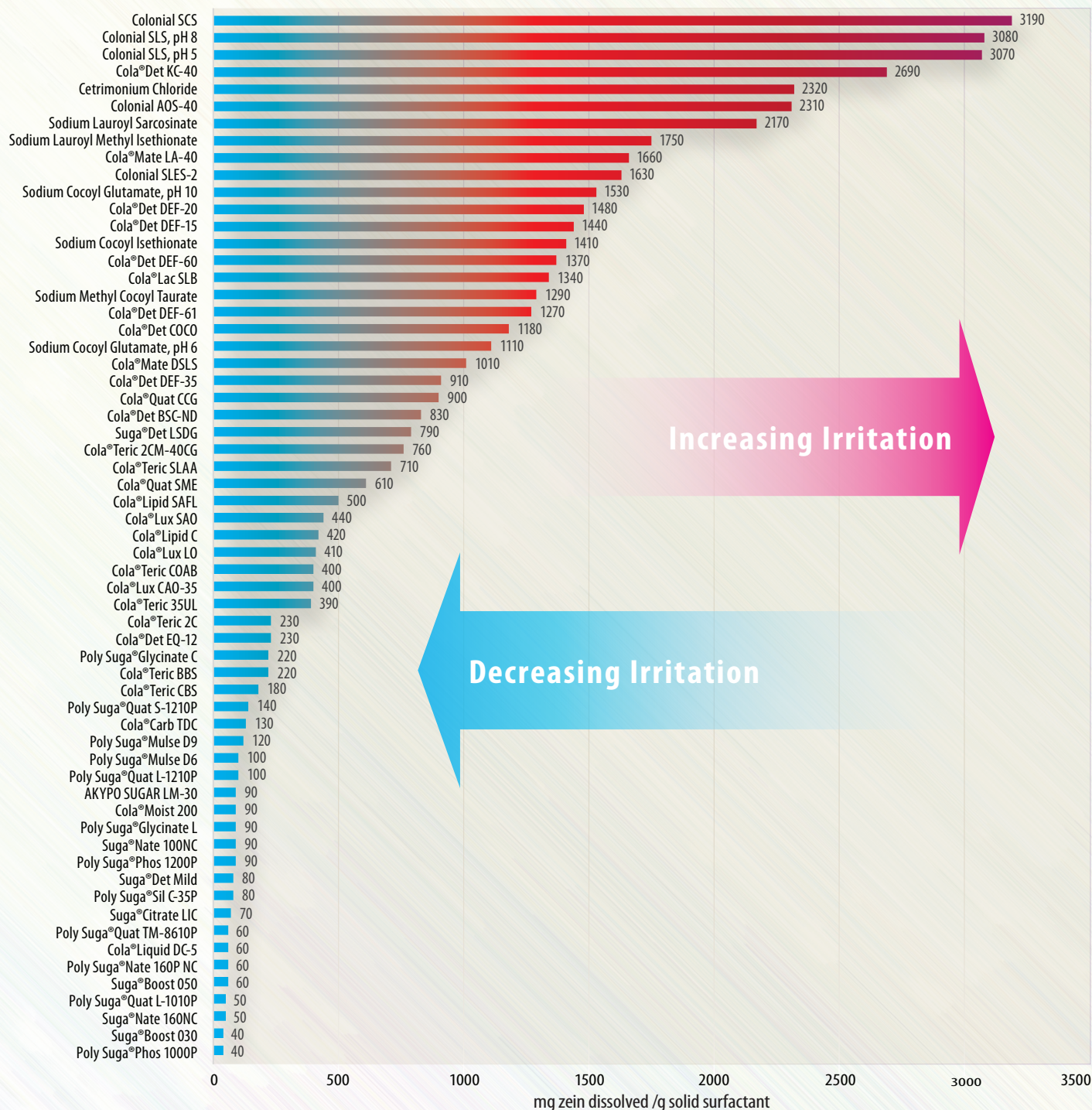
Vegan-compliant lab scale zein testing has been conducted on over 60 individual surfactant ingredients and commercial cleansing products, covering traditional chemistry as well as new innovations in all major surfactant types.



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## Results

## Irritation Potential by Zein Test





## 4. Conclusions

Common surfactants demonstrated typical trends with regards to irritation potential in the test. Of note, Cetrimonium Chloride tested somewhat lower irritation than typical, and some nonionic surfactants scored near zero, despite demonstrating some potential for irritation in other in vitro tests or in vivo. Ammonium salt surfactants did not demonstrate the reduction in irritation that could be described as “common knowledge” among formulators. Also, true amphoteric surfactants and some amino acid surfactants have the potential to demonstrate different ionic characteristics dependent on pH. So while Sodium Lauryl Sulfate (SLS) measured at pH 5 and 8 are effectively identical scores, (Di)Sodium Cocoyl Glutamate measured at pH 6 and 10 have substantially different scores. Most products were measured at their pH as supplied. Further investigation of the true amphoteric surfactants (amphoacetates, amphopropionates) at multiple pH values is warranted. Previous investigation of true soaps has also indicated that pH is an overriding factor in irritation in those products.

There is a significant amount of system error associated with this test, such that it is difficult to differentiate between very similar scores, especially at the low end of the chart. In some tests the product adsorbed to the protein with little or

no zein dissolving. This resulted in “negative” values, as the weight after drying was higher than before. This reinforces that while this is an effective screening test, further investigation is necessary to establish validated claims.

Surfactant combinations are well known to provide a synergistic reduction in irritation potential; the overall irritation tends to be lower than the predicted weighted average. By using newer generation amphoteric and nonionic surfactants (eg sultaine and alkyl glucoside), significant improvements can be realized when compared to more traditional secondary surfactants (betaine, alkanolamide). “Next generation” functionalized alkyl glucoside surfactants clearly demonstrate their favorable irritation profile relative to traditional anionic, cationic, and amphoteric surfactants.

Using an affordable and easy to use test methodology, we have quickly generated a large library of in chemico irritation data covering a wide range of frequently used surfactants. This will help formulators make better choices of specific surfactants and surfactant combinations, maximizing product performance while minimizing consumer risk. We will continue to generate data to provide the most robust body of data possible for formulators.

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2. Götte. *Skin compatibility of tensides measured by their capacity for dissolving zein protein*. In: Proc IV International Congress on Surface Active Substances, Brussels, Belgium, p 83–90 (1964).
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