

Raising preservative efficacy via glycol replacement

Zemea¹ bio-derived propanediol, manufactured by DuPont Tate & Lyle Bio Products, can be used to replace petroleum based glycols such as propylene glycol (PG), butylene glycol (BG) or glycerin in cosmetic and personal care formulations. It is currently being used as a humectant, emollient, and/or natural solvent in skin and hair care products and as a solvent for botanical extraction and dilution. It is also being used as a carrier for active ingredients, as an ingredient in natural preservative systems and for developing natural esters. Previously in this journal (September 2010) the results of various technical and consumer tests conducted to evaluate the performance of bio-derived propanediol as compared to traditional glycols and glycerin were reported. New testing recently conducted looked at the potential for bio-derived propanediol to boost the efficacy of preservatives in a personal care formulation.

Preservatives

A preservative is a natural or synthetic chemical that is added to products such as foods, cosmetics or pharmaceuticals to prevent spoilage. The primary reason preservatives are added to cosmetics and personal care formulations is to ensure the safety of these products for consumers. Microorganisms such as bacteria, yeasts and moulds are present in the air, in water and on human skin. They can cause irritation and infection when exposed to human skin and result in instability of the formulation including separation of the emulsion and bad odour. The use of preservatives in aqueous based cosmetics and personal care products prevents the growth of microorganisms.

Due to the potential health and safety risks for consumers, it is necessary for formulators to find a preservative system that will inhibit the growth of multiple microorganisms such as bacteria, yeasts and moulds. Formulation preservation is typically achieved at levels between 0.5% and 2% by weight of preservative.

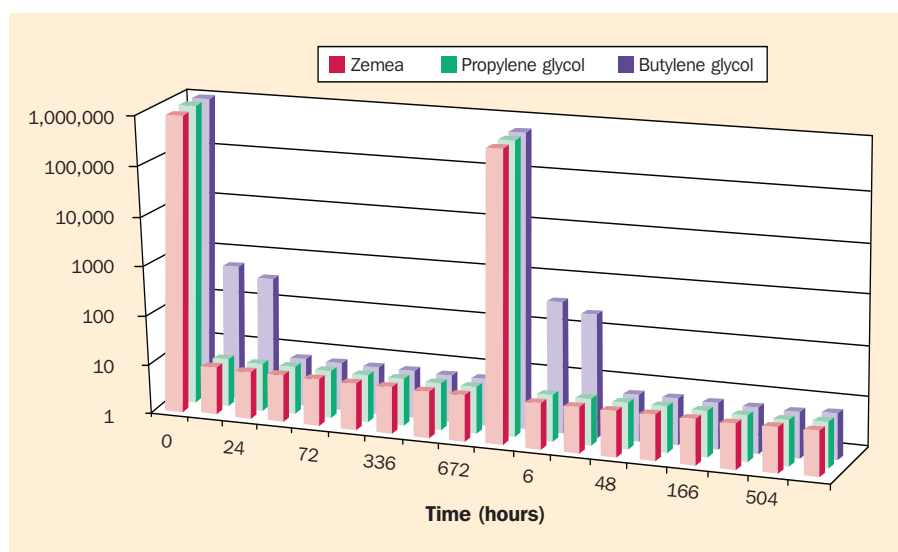


Figure 1: CTFA challenge test: Group III moulds and yeasts.

While preservative mechanisms and effectiveness may be similar, it is common for formulators to combine preservatives together in an attempt to create a synergy that can reduce the amount needed to effectively protect a product.

Over the last couple of years, consumers have been expressing concerns about the safety and use of preservatives in cosmetics and personal care products. Parabens, the most widely used preservative, have been singled out; and marketers have been touting paraben-free and/or preservative-free product claims

for some of their formulations. Ingredient suppliers have been developing new, natural preservative systems to meet the demand for natural product formulations and to provide alternatives to paraben-based preservatives.

The intent of this paper is to discuss the results of a recent study where the potential for boosting preservative efficacy by using bio-derived propanediol in an aqueous-based cosmetic formulation was studied using standardised microbiology guidelines, known as preservative efficacy testing or challenge tests.

Table 1: Formulation used in challenge test.

Ingredient	INCI Name	Weight, %
Water, deionised	Water	qs to 100%
Zemea propanediol	Propanediol	0 to 6.0
Xanthan gum	Xanthan gum	0.3
Liponate GC	Caprylic Capric Triglyceride	10.0
Sesame oil	Sesamum Indicum (Sesame) seed oil	5.0
Lipomulse 165	Glyceryl Stearate	2.0
Promulgen D	Cetearyl Alcohol and Ceteareth 20	1.5
DC 200-100	Dimethicone	1.0
NaOH /Citric acid (20% sol)	Sodium hydroxide/citric acid	qs to pH 5.0-6.0
Preservative*	Preservative*	(Table 2)

Table 2: Preservative systems used in the challenge test.

Preservative	INCI Name	Suggested weight %	Tested weight %	Zemea weight %			
Microcare PM3	Phenoxyethanol, Methylparaben, Propylparaben, Ethylparaben	0.3-0.7	0.15	0.0	2.0	4.0	6.0
Euxyl PE 9010	Phenoxyethanol, Ethylhexylglycerin	0.5-1.0	0.25	0.0	2.0	4.0	6.0
Neolone PE	Phenoxyethanol, Methylisothiazolinone	0.6	0.3	0.0	2.0	4.0	6.0
Jeecide CAP-4 Optiphen	Phenoxyethanol, Caprylyl glycol	0.5-1.5	0.25	0.0	2.0	4.0	6.0
Lexgard Natural	Glyceryl Caprylate, Glyceryl Undecylenate	1.0-1.5	0.5	0.0	2.0	4.0	6.0
Dermosoft 688 ECO	Anisic acid, Parfum	0.2	0.1	0.0	2.0	4.0	6.0
Geogard ULTRA	Gluconolactone, Sodium benzoate	1.0	0.5	0.0	2.0	4.0	6.0

CTFA challenge tests

Previously, neat glycol samples of bio-derived propanediol, propylene glycol and butylene glycol were compared using independent CTFA challenge tests for antifungal and antimicrobial properties. The testing was performed at Loricon Testing Service Inc., Keyport, New Jersey. The same procedure was used for each challenge test. The samples were inoculated with approximately 6×10^6 numbers of colonies and the numbers of colonies were counted periodically. After four weeks the inoculation was repeated.

Antifungal

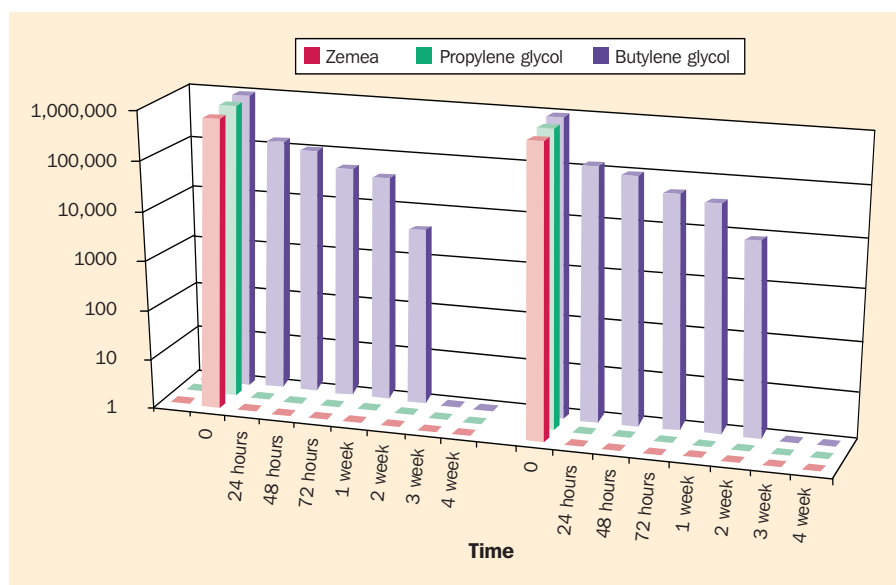
The first test evaluated the antifungal effectiveness of each glycol against Group I molds and yeasts. The moulds and yeasts used in this testing include *Aspergillus niger*, *Candida albicans*, blue/green penicillium and trichoderma.

Based on the results shown in Figure 1, it can be concluded that bio-derived propanediol has performance equal to PG and slightly better performance than BG after both the first and second inoculations.

Antimicrobial

The second test evaluated the antimicrobial effectiveness of each glycol against Group I, II and IV bacteria. The bacteria used in the testing include Group I: *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Enterobacter cloacae*, *Enterobacter gergoviae*; Group II: *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas cepacia*, *Flavobacterium* sp; and Group IV: bacterial isolates. Based on the results shown in Figure 2, it can be concluded that against Group I, bio-derived propanediol has equal performance to PG and significantly better performance than BG after both the first and second inoculations. Bio-derived propanediol also showed equal performance to PG and BG against Group II and IV bacteria.

Market feedback suggested that the bio-derived propanediol may help to

**Figure 2: CTFA challenge test: Group I bacteria.**

boost preservative efficacy when used in cosmetic and personal care formulations. Further testing was conducted and the results of the additional testing are the focus of this article.

The results are presented as three separate studies, but were based on similar experimental design and methods. Differences between the studies included varying the use level of bio-derived propanediol, further reductions in the amount of preservatives used, and the addition of butylene glycol.

Experimental design – A

Formulation

A generic oil-in-water skin care emulsion formula was chosen as the base material

and prepared by Cosmetech Laboratories, Inc., Fairfield, NJ (Table 1). The formula was prepared to minimise performance impact and allow measurement of preservative boosting effectiveness.

Preservatives

The preservatives listed in Table 2 were chosen to represent combinations commonly used for their effectiveness to protect products. The four phenoxyethanol-based and three natural-based systems were tested at one-half their recommended use level and evaluated in four separate emulsions with varying levels of the bio-derived propanediol (0.0 wt%, 2.0 wt%, 4.0 wt% and 6.0 wt%).

Table 3.

Organism	Inoculation	Incubation temperature
<i>Staphylococcus aureus</i> (ATCC#6538)	1×10^6 CFU/g	30-37°C
<i>Escherichia coli</i> (ATCC#8739)	1×10^6 CFU/g	30-37°C
<i>Pseudomonas aeruginosa</i> (ATCC#9027)	1×10^6 CFU/g	30-37°C
<i>Candida albicans</i> (ATCC#10231)	1×10^5 CFU/g	30-37°C
<i>Aspergillus niger</i> (ATCC#16404)	1×10^5 CFU/g	20-25°C

Table 4: Minimum percentage of Zemea needed to boost preservative efficacy.

	Gram-positive	Gram-negative	Gram-negative	Yeast	Mould
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>
Phenoxyethanol-based preservatives					
Microcare PM3 (0.15%)	2%	2%	2%	4%	2% (1 Log reduction)
Euxyl PE 9010 (0.25%)	4%	4%	2%	6%	2% (1 Log reduction)
Meolone PE (0.3%)	2%	2%	*	6%	2% (1 Log reduction)
Jeecide CAP-4 Optiphen (0.25%)	2%	2%	*	6%	2% (1 Log reduction)
Natural preservatives					
Lexgard Natural (0.5%)	*	*	*	*	2% (1 Log reduction)
Dermosoft 688 ECO (0.1%)	*	*	*	2%	2% (1 Log reduction)
Geogard ULTRA (0.5%)	*	*	*	2%	2% (1 Log reduction)

*Preservative levels provided sufficient reduction to <1.00 CFU/g without addition of Zemea.

Test methods & organisms

The challenge testing was conducted by Clinical Research Laboratories, Piscataway, NJ. The methods employed were CTFA Microbiology Guidelines, Section 20, M-3, A Method for Preservation Testing of Water Miscible Personal Care Products and USP 33, Section 61, Neutralization/Removal of Antimicrobial Activity.

Using the organisms listed in Table 3, the formulations were inoculated with approximately 1×10^6 bacteria per gram of product, 1×10^5 yeast cells per gram of product, or 1×10^5 mould spores per gram of product.

The microbial count was measured at 1, 2 and 7 days to determine the

survivability of the microorganisms in the preserved test formulations.

Acceptance criteria

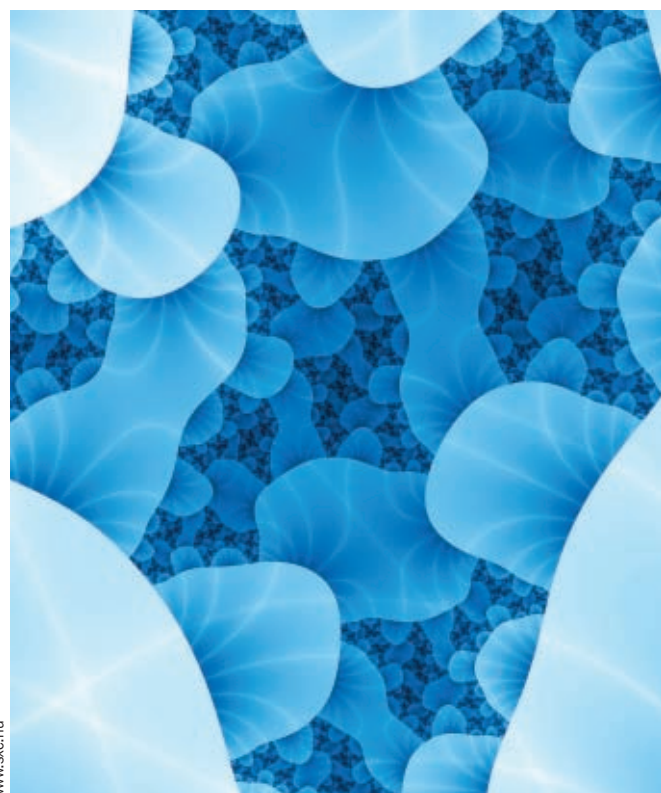
In this type of testing, the preservative is considered effective in the sample examined if:

- The concentrations of viable bacteria demonstrate no less than a 3 Log reduction (99.9%) from the initial count at 7 days, and no increase for the duration of the test period.
- The concentration of viable yeast and moulds demonstrate no less than a

1 Log reduction (90.0%) from the initial count at 7 days, and no increase for the duration of the test period.

Results

Table 4 shows the minimum percentage of bio-derived propanediol needed to boost the preservatives efficacy when used at one-half their recommended use level. These percentages are based on the concentrations of viable bacteria and yeasts reduced to <1.00 CFU/g at Day 7, and concentrations of viable moulds with a 1 Log reduction at Day 7.



Use of preservatives in personal care products prevents the growth of microorganisms.

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Table 5: Challenge test results at Day 7 using 6% Zemea + 0.25% preservative levels.

	Gram-positive	Gram-negative	Gram-negative	Yeast	Mould
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>
Phenoxyethanol-based preservatives					
Microcare PM3 (0.075%)	<1.00	<1.00	<1.00	DNP	2 Log reduction
Euxyl PE 9010 (0.125%)	<1.00	<1.00	<1.00	DNP	2 Log reduction
Neolone PE (0.15%)	<1.00	<1.00	<1.00	DNP	<1.00
Jeecide CAP-4 Optiphen (0.125%)	<1.00	<1.00	<1.00	DNP	<1.00
Natural preservative					
Lexguard Natural (0.25%)	<1.00	<1.00	<1.00	3 Log reduction	DNP
Organism count at Day 7, CFU/g					

- Bio-derived propanediol worked well with the phenoxyethanol-based preservatives and boosted the preservative efficacy for gram-positive, gram-negative, and yeast organisms.
- Bio-derived propanediol consistently boosted the efficacy of each preservative tested with *Aspergillus niger*.
- Bio-derived propanediol worked well with the natural based preservatives and boosted the preservative efficacy for yeast and moulds.
- Bio-derived propanediol may allow the use of less preservative in formulations while providing additional performance benefits such as no skin irritation, increased humectancy and excellent aesthetics.
- Bio-derived propanediol is not a preservative nor is it considered an active ingredient.

Experimental design – B

Consistent with experimental design A, the results of a second CTFA challenge study are described. The base emulsion formula (Table 1) and preservative systems (Table 2) were used with minor changes listed for both. The Dermosoft 688 ECO and Geogard ULTRA were excluded for this study.

The study evaluated the base emulsion formula (Table 1) with a constant level of 6% bio-derived propanediol and with the

preservative systems tested at one-quarter their recommended use level. Table 5 shows the challenge test results at Day 7.

Results

- Bio-derived propanediol worked well with the lower preservative levels and boosted their efficacy for Gram-positive and Gram-negative organisms.
- Bio-derived propanediol consistently boosted the efficacy of the phenoxyethanol-based preservatives tested with *Aspergillus niger*.

Experimental design – C

Consistent with experimental design A, the results of a third CTFA challenge study are described. The base emulsion formula (Table 1) used in this study replaced the bio-derived propanediol with butylene glycol and the preservative system levels were used as described in Table 2. The Dermosoft 688 ECO and Geogard ULTRA were excluded for this study.

The study evaluated the base emulsion formula (Table 1) with a constant level of 4% butylene glycol and with the preservative systems used at one-half their recommended use level. Table 6 shows the challenge test results at Day 7.

Results

- 4% butylene glycol did not work well with the preservative levels used in this study.

- 4% butylene glycol inconsistently boosted the efficacy with Gram-negative and yeast organisms.

Conclusion

Zemea bio-derived propanediol is the world's first 100% natural glycol replacement approved by ECOCERT and certified by the Natural Products Association. With its skin-friendly performance, including no irritation, enhanced moisturisation and excellent aesthetics, Zemea is seeing rapid adoption around the world in skin care, hair care, deodorants, fragrances, and other cosmetic and personal care products. Based on rapidly renewable resources, Zemea can successfully replace petroleum-based glycols or glycerin in many natural and traditional personal care formulations. Formulators may be able to reduce the amount of preservatives used in their formulations with the inclusion of this natural glycol replacement.



Acknowledgements

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Footnote

- 1 Zemea® propanediol is a registered trademark of DuPont Tate & Lyle Bio Products LLC.

Table 6: Challenge test results at Day 7 using 4% butylene glycol + 0.5% preservative levels.

	Gram-positive	Gram-negative	Gram-negative	Yeast	Mould
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>
Phenoxyethanol-based preservatives					
Microcare PM3 (0.15%)	DNP	DNP	DNP	DNP	DNP
Euxyl PE 9010 (0.25%)	DNP	DNP	DNP	DNP	DNP
Neolone PE (0.3%)	DNP	DNP	<1.00	1 Log reduction	DNP
Jeecide CAP-4 Optiphen (0.25%)	DNP	DNP	4 Log reduction	DNP	DNP
Natural preservative					
Lexguard Natural (0.5%)	<1.00	<1.00	DNP	2 Log reduction	DNP
Organism count at Day 7, CFU/g					