



Technical Data Sheet

1. INCI composition

5. Properties

Benzyl alcohol Glyceryl caprylate Glyceryl undecylenate

2. General description

TRIstat ECO is a broad spectrum preservative blend based on natural identical and natural-derived ingredients, for the protection of natural and traditional cosmetics in a wide range of pH. It is a synergistic combination of benzyl alcohol with glyceryl monoesters that boosts the preservative action and add emolliency. TRIstat ECO is gentle to the skin and active against bacteria, yeasts & moulds. Colourless and with very mild odour, it is effective in the pH range from 4 to 8.

TRIstat ECO is globally approved and suitable for wide range of personal care products for rinseoff & leave-on. It is permitted for the preservation of natural & organic cosmetics according to the major certification standards (Ecocert, BDIH, Soil Association, ICEA, COSMOS Standard, Natrue, NPA).

3. Specification data

Appearance: Clear colourless to pale

vellow liquid

Slight characteristic Odour:

Benzyl alcohol: 43.0-47.0% **Glyceryl monoesters:** 38% min.

Density (@20°C): 1.026-1.036 g/mL Refractive index (@20°C): 1.4820-1.5020 **Heavy metals:** 10 ppm max.

Shelf life: 2 years in original packing

4. Other properties

pH (0.5% water dispersion):

Approx. 5 Less than 0°C Freezing point:

TRIstat ECO is an innovative preserving system that combines the well-established and natural-identical cosmetic preservative Benzyl alcohol with two high purity monoesters 100% natural-derived. Glyceryl Glyceryl undecylenate caprylate and possess themselves antibacterial and antifungal activity respectively, and enhance the activity of benzyl alcohol. As a result, TRIstat ECO is a synergistic preservative system with broad spectrum activity suitable to control microbial growth in cosmetics with no need of additional preservatives.

Benzyl alcohol is an aromatic alcohol with a mild and sweet odour, it is a natural constituent of some essential oils (jasmine, hyacinth, ylang-ylang), many edible fruits and green/black tea.

It is largely used as preservative in cosmetics, foods and pharmaceuticals, as component of fragrances and also as a local anaesthetic.

Benzyl alcohol is active primarily against bacteria and molds. The mechanism of action is the disruption of cell membrane barrier by solubilization of lipids, the enhancement of the permeability of microbial cells to other antimicrobials and the inhibition of essential enzymes involved in the Kreb's cvcle1.

It is worldwide approved and mainly used in combination with other preservatives, mav substitute highly effective but controversial preservatives.

Glyceryl caprylate and Glyceryl undecylenate are monoesters obtained from the reaction of glycerol with caprylic acid and undecylenic acid respectively, both 100% of vegetal origin. Glyceryl esters are present in natural oils and in the metabolism of living systems. They are widely used in cosmetics, foods & pharmaceuticals as possess multiple beneficial properties as co-emulsifiers, emollients, wetting agents and solubilizers.

Additionally esters of medium chain fatty acids with C8 to C12 chain length like glyceryl caprylate, glyceryl caprate, glyceryl undecylenate & glyceryl laurate have antimicrobial activity². These C8-C12 esters have the suitable length to enter, destabilize the microbial membrane and destroy microbial cells3.

It is also known that the high purity monoesters, which have higher levels of monoglycerydes, have greater biocidal activity4.



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7. Stability

Glyceryl monoesters are ideal examples of multifunctional ingredients which act as co-emulsifiers, humectants, refatting agents and skin-feel enhancers.

Besides, they exerts antimicrobial action and act as preservative boosters that allows preservatives to enter the microbial cell more effectively. Therefore glyceryl esters become part of the preservative system and reduce preservative requirements.

As a synergistic combination of an alcohol & glyceryl esters, *TRIstat ECO* is an efficient antimicrobial system not pH-dependent with low irritant potential that represents a new interesting option in cosmetic preservation.

TRIstat ECO is also synergistic with other ingredients of formulations like chelating agents, antioxidants, surfactants and organic acids, this synergism further reduces the use level in the finished cosmetic.

The multiple benefits of *TRIstat ECO* allow formulators to create safe self-preserving cosmetics with reduced content of traditional preservatives according to the "hurdle technology"⁵. This is a modern holistic approach to preservation, largely used in foods, making use of several hurdles that create a hostile environment to microorganisms and inhibit microbial metabolism and reproduction.

6. Solubility

TRIstat ECO is very soluble in alcohol, glycerine, glycols and polar oils, very slightly soluble in water. The solubility of TRIstat ECO at 20°C in common cosmetic solvents is showed below (expressed as weight in grams of TRIstat ECO dissolved in 100 g of solution):

Solvent	%w/w
Water	< 0.1%
Ethanol	> 50
Glycerin	> 50
Propylene glycol	> 50
Ethylhexyl palmitate	> 50
Octyldodecanol	> 50
Mineral oil	1.2

TRIstat ECO may tolerate process heating to 80°C for approx. 30 minutes, anyway prolonged high thermal exposure are not recommended and the use of the lowest possible temperature is suggested.

It should be stored in the tightly closed original container at temperature to 25°C, protected from direct sunlight and frost. The product is stable to temperature near 0°C.

8. Antimicrobial Activity

The activity of *TRIstat ECO* against microbial contaminants was determined following the *Minimum Inhibitory Concentration (MIC)* and *Minimum Biocidal Concentration (MBC)* method⁶.

The test was conducted using the standard panel of microorganisms (bacteria and fungi) used in pharmacopeial tests. Microbial suspensions of microorganisms derived from ATCC were prepared in suitable nutrient broths. Serial dilutions of the preservative were carried out aseptically in microtiter plates. Each well was inoculated with a single culture to the concentration of 10^{5} - 10^{6} cells/mL. The plates were incubated at 35°C for 48 hours for bacteria and at 25°C for 5 days for fungi.

The *minimum inhibitory concentration (MIC)* provides the bacteriostatic/fungistatic activity, that is the ability to inhibit the reproduction of microorganisms. The *MIC value* is the lowest concentration which inhibits the visible growth of test organisms after 48 hours for bacteria, 72 hours for yeasts and 5 days for molds.

The *minimum biocidal concentration (MBC)* gives the bactericidal/fungicidal activity, which defines the ability to kill microorganisms after a contact time of 48 hours with the antimicrobial. This is obtained by plating an aliquot of each dilution without visible growth into a suitable solid nutrient media.

The <u>MBC value</u> is the lowest concentration of preservative that killed more than 99.9% of the initial concentrations of microorganisms.



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Tab 6.1: Inhibitory (MIC) and biocidal (MBC) activity of TRIstat ECO in ppm

Test organis (≅ 106CFU/m		MIC	МВС
Gram-negative bacter	ria		
Escherichia coli	ATCC 8739	3000	4000
Pseudomonas aeruginosa	ATCC 9027	3000	<i>5000</i>
Gram-positive bacter	ia		
Staphylococcus aureus	ATCC 6538	2500	<i>3500</i>
Yeasts			
Candida albicans	ATCC 10231	1000	4000
Molds			
Aspergillus brasiliensis	ATCC 16404	2500	10000

TRIstat ECO has bacteriostatic and fungistatic effect at levels of 0.3%. Additionally it is bactericidal at

0.5%, fungicidal against the yeast *C. albicans* at 0.4% and fungicidal against the moulds *A. Niger* at 1.0%.

 $TRIstat\ ECO$ is also effective in reducing and inhibiting the growth of $P.\ acnes$ at concentration as low as 0.1%.

NOTE: The above MIC/MBC values estimate the antimicrobial activity of preservatives in culture media. The optimal use levels to be used in cosmetic products should be determined by challenge testing. On demand, our microbiological laboratory may provide the appropriate support.

9. Challenge tests

The antimicrobial activity of *TRIstat ECO* in different cosmetic formulations was evaluated by challenge testing, using a modified *European Pharmacopoeia* method (5.1.3 Efficacy of antimicrobial preservation). The tests were performed using the following microorganisms:

- E. coli ATCC 8739
- P. aeruginosa ATCC 9027
- S. aureus ATCC 6538
- C. albicans ATCC 10231
- A. brasiliensis ATCC 16404.

Cosmetic samples with different levels of preservative were challenged with mixed inocula of bacteria and fungi separately, to reach microbial levels of not less than 10^6 cfu/g for bacteria and 10^5 cfu/g for fungi. These samples were tested for microbial count at 0, 2, 7, 14, 21, 28 days.

Microbial counts were performed on 1 g of test sample serially diluted and plated in suitable solid media; plates are incubated at 35°C for bacteria and at 25°C for fungi, then the number of colonies per gram (cfu/g) are counted.

The preservative is considered effective and the products tested are *adequately preserved* when the following requirements are fulfilled:

- The concentration of viable bacteria shows not less than 2 Log reduction (\geq 99%) at 2 days from the initial count and not less than 3 Log reduction (\geq 99.9%) at 7 days.
- The concentration of the yeast *C. albicans* & the mould *A. brasiliensis* demonstrates not less than 2 Log reduction (99%) at 14 days.
- No subsequent increase in the number of viable microorganisms is observed for the duration of the test period.

The composition of the formulations tested (natural o/w emulsion & mild shower bath) as well as the results obtained are showed in the following tables and graphs.



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Natural O/W emulsion

INCI Name	Weigh	t %	
Arachidyl glucoside, Arac	chidyl alco	ohol	
Behenyl alcohol			5.0
Caprylic/capric triglyceri	de		4.0
Cetyl alcohol			4.0
Glycerin			4.0
Octyl palmitate			3.0
Allantoin			0.3
Sodium phytate	0.1	0.1	0.2
Sodium hydroxide 10%			q. s. Water
			to 100
TRIstat ECO 0 Final pH 6.0	1.4	1.5	1.4

Results:

The *unpreserved* emulsion supported very high levels of viable organisms for all test period.

The formulation with **1.4% TRIstat ECO** lowered bacteria by more than 2 Log (>99%) in 2 days and fully inactivated them in 7 days. *C. albicans* was fully inactivated within 7 days. *A. brasiliensis* was reduced by 1 Log (90%) in 14 days, by more than 2 Log in 21 days and more than 3 Log (>99.9%) at the end of the test. According to the Ph. Eur. criteria of acceptance, this formulation was judged poorly preserved against A. brasiliensis and adequately preserved against bacteria and *C. albicans*. The formulation with **1.5% TRIstat ECO** totally inactivated bacteria and *C. albicans* in 2 days.

A. brasiliensis was reduced by more than 2 Log (>99%) in 7 days, more than 3 Log in 14 days and fully inactivated in 21 days. This formulation was adequately preserved against all test organisms.

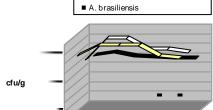
The formulation with **1.4% TRIstat ECO + 0.2% Sodium phytate** showed greater than 4 Log (>99.99%) reduction of bacteria in 2 days and complete inactivation in 7 days. *C. albicans* was fully inactivated within 2 days. *A. brasiliensis* w a s reduced by more than 2 Log in 7 days, more than 3 log in 14 days and completely inactivated in 21.

In conclusion, the use level of 1.5% TRIstat ECO provided satisfactory and broad spectrum coverage to a typical O/W emulsion, according to the Ph. Eur. requirements. Interestingly the combination of 1.4% TRIstat ECO with the chelating agent Sodium phytate at 0.2% significantly increases the antimicrobial activity and proves the synergistic effect with chelating agents, useful to reduce the final use levels of TRIstat ECO in cosmetics.

Preservation efficacy on natural o/w emulsion unpreserved

Strains	time 0	2 days	7 days	14 days	21 days	28 days	Results
E. coli P. aeruginosa S. aureus	4.6.106	>107	>107	>107	3.6·10 ⁶	2.2·10 ⁶	Failed
C. albicans A. brasiliensis	2.8·10 ⁵ 1.7·10 ⁵	>10 ⁷ 3.5·10 ⁵	>10 ⁷ 1.2·10 ⁵	6.5·10 ⁶ 8.8·10 ⁴	3.4·10 ⁶ 7.8·10 ⁴	1.7·10 ⁶ 7.5·10 ⁴	Failed Failed

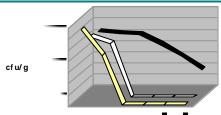
■ E. coli + P. aeruginosa + S. aureus



C. albicans

Preservation efficacy on natural o/w emulsion with 1.4% TRIstat

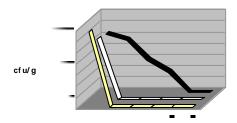
Strains	time 0	2 days	7 days	14 days	21 days	28 days	Results
E. coli P. aeruginosa S. aureus	4.6·10 ⁶	2.3·10 ⁴	<1N	<1N	∠ 10	∠ 1∩	rasseu
C. albicans A. brasiliensis		6.7·10 ⁴ 1.1·10 ⁵		<10 1.2·10 ⁴	<10 1.1·10 ³	<10 70	Passed Failed



Preservation efficacy on natural o/w emulsion with 1.5% TRIstat

ECO

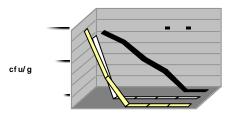
Strains	time 0	2 days	7 days	14 days	21 days	28 days	Results
E. coli P. aeruginosa S. aureus	4.6·10 ⁶	<10	<10	<10	<10	<10	Passed
C. albicans A. brasiliensis	2.8·10 ⁵ 1.7·10 ⁵	<10 5.4·10 ⁴	<10 7.9·10 ²	<10 70	<10 <10	<10 <10	Passed Passed



Preservation efficacy on natural o/w emulsion with 1.4% TRIstat ECO

+ 0.2% Sodium phytate

Strains	time 0	2 days	7 days	14 days	21 days	28 days	Results
E. coli P. aeruginosa S. aureus	4.6·10 ⁶	3.1·10 ²	<10	<10	<10	<10	Passed
C. albicans A. brasiliensis	2.8·10 ⁵ 1.7·10 ⁵	<10 2.1·10 ⁴	<10 6.6·10 ²	<10 60	<10 <10	<10 <10	Passed Passed







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Mild Shower Bath

Capryl/caprylyl glucoside 64%	20.0
Sodium lauroyl sarcosinate 30%	15.0
Glycerin	3.0
Sodium Chloride	0.9
Allantoin	0.2
Phytic acid 50%	0.2
Citric acid	q. s.
Water TRIstat ECO 0 1.2 1.4	to 100

Final pH 5.0

Results:

The *unpreserved* mild shower bath was susceptible to bacterial as well as fungal contamination and supported microbial growth for all the test period.

bacteria and *C. albicans* within 2 days with no further increase.

A. niger was reduced by >1 Log (>90%) in 2 days, >2 Log (>99%) in 7 days and was completely inactivated in 21 days.

The formulation with 1.2% TRIstat ECO can be considered as <u>adequately preserved</u> against all test organisms, according to Ph. Eur. criteria of acceptance.

The formulation with **1.4% TRIstat ECO** showed further reduction of *A. niger* by more than 2 Log in 2 days.

The formulation with **1.6% TRIstat ECO** totally inactivated *A. niger* in 14 days.

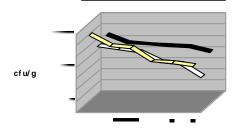
In conclusion, the use level of 1.2% TRIstat ECO can provide satisfactory and broad spectrum protection to a typical surfactant-based cosmetic according to the Ph. Eur. requirements. Concentration of 1.4% and 1.6% showed higher inactivation rate of A. brasiliensis.

NOTE: Value <10 is the limit of detection of plate count method and

Preservation efficacy on mild shower bath unpreserved

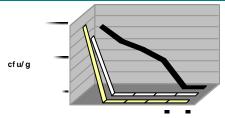
Strains	time 0	2 days	7 days	14 days	21 days	28 days	Results
E. coli P. aeruginosa S. aureus	4.6·10 ⁶	5.5·10 ⁵	3.4·10 ⁵	2.1.104	1.6·10 ⁴	7.9·10³	Failed
C. albicans A. brasiliensis		6.7·10 ⁴ 5.4·10 ⁴		4.5·10³ 2.8·10⁴	3.0·10 ³ 2.1·10 ⁴	2.1·10² 8.5·10³	Failed Failed





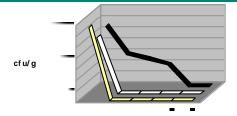
Preservation efficacy on mild shower bath with 1.2% TRIstat

Strains	time 0	2 days	7 days	14 days	21 days	28 days	Results
E. coli P. aeruginosa S. aureus	4.6·10 ⁶	<10	<10	<10	<10	<10	Passed
C. albicans A. brasiliensis	1.2·10 ⁵ 2.6·10 ⁵	<10 1.1·10 ⁴	<10 2.4·10 ³	<10 2.3·10 ²	<10 <10	<10 <10	Passed Passed



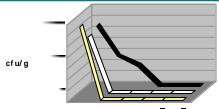
Preservation efficacy on Mild Shower Bath with 1.4% TRIstat ECO

	Strains	time 0	2 days	7 days	14 days	21 days	28 days	Results
d	E. coli P. aeruginosa S. aureus	4.6·10 ⁶	<10	<10	<10	<10	<10	Passed
	C. albicans A. brasiliensis	1.2·10 ⁵ 2.6·10 ⁵			<10 90	<10 <10	<10 <10	Passed Passed



Preservation efficacy on Mild Shower Bath with 1.6% TRIstat ECO

Strains	time 0	2 days	7 days	14 days	21 days	28 days	Results
E. coli P. aeruginosa S. aureus	4.6.106	<10	<10	<10	<10	<10	Passed
C. albicans	1.2·10 ⁵	<10	<10	<10	<10	<10	Passed
A. brasiliensis	2.6·10 ⁵	$4.7 \cdot 10^{2}$	70	<10	<10	<10	Passed
					-		







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10. Applications

TRIstat ECO is a preservative system with broad spectrum activity for the protection of natural & organic cosmetics as well as standard and in particular high quality skin care cosmetics.

The components of *TRIstat ECO* are natural-compliant cosmetic ingredients permitted in any cosmetic applications that meets the requirements of the major standards for the certification of natural & organic cosmetics like Ecocert (France), Soil Association (UK), BDIH (Germany), ICEA (Italy), COSMOS (EU), NPA (US) and NaTrue.

TRIstat ECO is effective in the pH range from 4.0 to 8.0, a considerably broad pH that overcomes the lack of activity in neutral and slightly alkaline formulations of other preservatives like organic acids.

TRIstat ECO is a clear colourless liquid, with little pleasant odour, that does not change the organoleptic properties of cosmetics. It is compatible with most cosmetic ingredients and is fully effective in anionic, non-ionic and cationic systems.

The addition to emulsions can cause a reduction of viscosity, thus the stability should be checked and adjusted with thickening agents or waxes if needed.

It is synergistic with organic acids (citric acid, lactic acid), chelating agents (phytic acid trisodium ethylenediamine disuccinate, tetrasodium glutamate diacetate), antioxidants (tocopherol), essential oils and fragrances, therefore represents an ideal multifunctional system to develop safe self-preserving cosmetics with reduced preservative content, according to the principles of Hurdle technology. This is a system approach that make an intelligent use of preservation factors (hurdles) including low pH, low water activity, multifunctional agents and protective packaging to achieve mid preservation⁵.

TRIstat ECO is effective in reducing and inhibiting the growth of *P. acnes*, consequentely can be a useful

addition in anti-acne products, and in general in tonics, shampoos, creams & lotions cosmetics for the treatment of greasy & impure skin.

TRIstat ECO can be used in wide range of cosmetics for rinse-off and leave-on. Due to its miscibility in alcohols and oils it can be easily incorporated in surfactant-based products, emulsions and oils.

It is practically not soluble in water, but it is dispersible in aqueous systems where can be dissolved with the addition of solubilising agents.

Even if heating to 80°C is tolerated, the prolonged exposition to high heating process is not suggested and the use of the lowest possible manufacturing temperature is recommended.

TRIstat ECO can be incorporated in aqueous & hydroalcoholic formulations and clear gels with the addition of solubilizers upon stirring at room temperature or with gentle heating to accelerate the dissolution.

In *surfactant-based systems* can be easily incorporated at room temperature into the surfactant blends before the addition of the other components.

In *emulsions* can be added in the oil phase, in the water phase or after the emulsification, preferably during the cooling stage at temperature below 50°C. It is compatible and freely soluble in *oil-based formulations*.

The typical recommended use levels are within 1.0-1.6%, which can be increased to 2.0% in more complex and contaminable formulations.

Formulation types	Applications	% use levels
Aqueous formulations	hair lotions & gels face serums tonics gel facial masks shaving gels aftersun gels body & foot gels baby lotions	1.2-1.8
Surfactant-based formulations	shower gels bubble baths shampoos hair conditioners mousses shaving foams hand cleaners intimates baby baths & shampoo	1.0-1.6
Emulsions	face lotions & creams body lotions & creams baby lotions & creams suncare lotions & creams shaving lotions & creams antiacne lotions & creams hand & foot creams foundations	1.2-2.0
Oil-based formulations	baby oils bath oils body & foot oils sunscreens & suntans	0.8-1.2
Powders & anhydrous	massage oils bath powders make-up powders muds	0.8-1.4
Raw materials	surfactants blends vegetal extracts	0.8-1.8

NOTE: The above use levels are only general indications. The exact use concentration is related to many factors including raw materials quality, manufacturing hygiene, formula composition and final packaging. The optimal amount of preservative should be determined by challenge testing in the specific products.



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11. Toxicological information

The components of *TRIstat ECO* are well established cosmetic ingredients, recognised as safe for any personal care applications and permitted at use levels up to 2.2% *TRIstat ECO* according to the EU cosmetic regulation. The safety of all the ingredients of *TRIstat ECO* for cosmetic use was reviewed by the Expert Panel of the US Cosmetic Ingredient Review (CIR).

Benzyl alcohol 7 is widely used in cosmetics as fragrance, solvent and preservative. In EU its use as a preservative is restricted to the maximum concentration of 1%, while it is not restricted in Japan. It is classified as fragrance allergens. Benzyl alcohol is approved as food & pharma additive.

Glyceryl caprylate⁸ and glyceryl undecylenate⁵ are well-established cosmetic raw materials and are generally considered to present no special hazards.

A clinical study by occlusive patch test showed that

TRIstat ECO is not irritant to human skin.

Based on these assessments, *TRIstat ECO* is considered as a safe cosmetic additive at the recommended use concentrations.

TRIstat ECO does not contain genetically modified material, BSE-related material and has not been tested on animals.

12. Regulatory status

TRIstat ECO is approved as cosmetic preservatives in EU, USA, Brazil, China & ASEAN countries to the maximum concentration of 2.2% in all type of cosmetics.

In Japan the components of TRIstat ECO are not recognised as preservatives and are permitted without restrictions.

13. Storage

TRIstat ECO should be stored in the well closed original container at temperature below 25°C, protected from direct sunlight and frost. Containers once opened should be firmly reclosed.

At these conditions its minimum shelf-life is two years from the manufacturing date.







INCI name:

ОН

Benzyl alcohol

Empirical formula: C7H8O Molecular weight: 108.1

CAS number: 100-51-6 EC name: Benzyl alcohol EC number: 202-859-9

OH HO O-

INCI name:

Glyceryl caprylate

Empirical formula: C₁₁H₂₂O₄ monoester

CAS number: 26402-26-6 EC name: Octanoic acid,

with 1,2,3propanetriol

Molecular weight: 218.29

EC number: 247-668-1

HOOH

INCI name:

Glyceryl undecylenate

Empirical formula: C₁₄H₂₆O₄

CAS number: 65684-27-7

EC name: Undecylenic acid, monoester with 1,2,3-

propanetriol

Molecular weight: 258.35

EC number: 236-935-8

15. References

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⁴ J. Kabara (1997) Fatty acid and esters as multifunctional

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preservatives requirements. In "Insights into cosmetic microbiology" pp. 89-96. Allured Books.

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⁷ Cosmetic Ingredient Review (2001) Final report on the safety assessment of benzyl alcohol, benzoic acid and sodium benzoate. International Journal of toxicology; 20 (3), pp. 23-50.

⁸ Cosmetic Ingredient Review (2004) Final report of the amended safety assessment of Glyceryl Laurate, Glyceryl Laurate SE, Glyceryl Laurate/Oleate, Glyceryl Adipate,

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Glyceryl Alginate, Glyceryl Arachidate, Glyceryl Arachidonate, Glyceryl Behenate, Glyceryl Caprylate, Glyceryl Caprylate, Glyceryl Caprylate, Glyceryl Caprylate, Glyceryl Undecylenate. International Journal of Toxicology; 23, pp. 55-94.

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