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(54) Title: MICROBIOCIDAL COMPOSITION

(57) Abstract: A microbiocidal composition comprising: a non-halogenated 2-alkyl-3-isothiazolone, and chlorphenesin and/or an alkane diol, wherein the alkyl group in the non-halogenated 2-alkyl-3-isothiazolone comprises 1 to 4 carbon atoms, the alkane diol comprises from 5 to 12 carbon atoms, and the hydroxyl groups of the alkane diol are vicinally substituted.

WO 2007/007080 A1

- 1 -

Microbiocidal Composition

The present invention relates to microbiocidal compositions, in particular microbiocidal compositions for use in cosmetic formulations.

It is desirable for cosmetic formulations to have a long shelf life. However, cosmetic formulations tend to degrade over time owing to bacterial or fungal growth in the formulation. There is therefore a desire to produce formulations that have an improved resistance to microbial attack, both from bacteria and fungi.

While chlorinated isothiazolones have been used in cosmetic formulations and are effective in relatively small concentrations, they have been found to be unstable with variations in pH and/or temperature. The chlorinated isothiazolones may also trigger allergies and this further restricts their use. Moreover, they may be incompatible with some cosmetic raw materials, especially raw materials which act as nucleophiles or reducing agents.

It is an object of the present invention to overcome, or at least mitigate, some of the problems associated with the compositions of the prior art.

Accordingly, the present invention provides a microbiocidal composition comprising:

a non-halogenated 2-alkyl-3-isothiazolone, and
chlorphenesin and/or an alkane diol,
wherein the alkyl group in the non-halogenated 2-alkyl-3-isothiazolone comprises 1 to 4 carbon atoms,
the alkane diol comprises 5 to 12 carbon atoms, and

-2-

the hydroxyl groups of the alkane diol are vicinally substituted.

Non-halogenated 2-alkyl-3-isothiazolones may act as biocidal agents. However, when used alone their performance in preventing growth of bacteria and fungi is relatively poor in comparison with an equivalent concentration of chlorinated isothiazolones.

However, non-halogenated 2-alkyl-3-isothiazolones have unexpectedly been found to be relatively stable to variations in pH and/or temperature compared to chlorinated isothiazolones. Furthermore, non-halogenated 2-alkyl-3-isothiazolones have been found to be more compatible than chlorinated isothiazolones with the raw materials typically present in cosmetic formulations.

It has also surprisingly been found that the combination of the non-halogenated 2-alkyl-3-isothiazolone and the alkane diol results in a synergistic effect in preventing microbial growth. As a consequence, a lower concentration of the non-halogenated isothiazolone may be used to effectively prevent microbial growth.

It has also been surprisingly found that the combination of a non-halogenated 2-alkyl-3-isothiazolone and chlorphenesin has a synergistic effect in preventing microbial growth.

The combination of the 2-alkyl-3-isothiazolone, chlorphenesin and the alkane diol results in a particularly effective microbiocidal composition in which all the ingredients have been found to act synergistically, i.e. the biocidal effect is greater than would be expected purely on the efficacy of the components alone.

- 3 -

Preferably, the 2-alkyl-3-isothiazolone and the alkane diol are present in the composition in a ratio (wt.%) range of from 1:3 to 1:400, more preferably of from 1:10 to 1:50, still more preferably of from 1:15 to 1 to 30. The 2-alkyl-3-isothiazolone and the alkane diol may be present in the composition in a ratio (wt.%) range of from 1:25 to 1:48, still more preferably from 1:30 to 1:45.

Preferably, the 2-alkyl-3-isothiazolone and the chlorphenesin are present in the composition in a ratio (wt.%) range of from 10:3 to 1:400, more preferably of from 1:10 to 1:50, still more preferably from 1:15 to 1:45, more preferably 1:15 to 1:30.

The ratio of 2-alkyl-3-isothiazolone:alkane diol:chlorphenesin may be in the range of from 1:10:10 to 1:20:20 (i.e. varying independently the amounts of alkane diol and/or chlorphenesin between their preferred upper and lower limits relative to the amount of isothiazolone).

The alkyl group in the 2-alkyl-3-isothiazolone may be substituted or non-substituted and may be branched or linear. The alkyl group may be substituted with one or more of the following: hydroxyl, cyano, alkylamino, carboxy, carboalkoxy and alkylthio.

Preferably, the non-halogenated 2-alkyl-3-isothiazolone is 2-methyl-3-isothiazolone.

It has been found by the inventors that different alkane diols exhibit different biocidal efficacies in combination with 2-alkyl-3-isothiazolones.

- 4 -

The alkane diol may comprise 5 to 12 carbon atoms. The alkane diol may comprises 5 to 10 carbon atoms, more preferably 6 - 8 carbon atoms, which may be in a linear or branched chain, preferably a linear chain. Preferably the alkane diol comprises 6 or more carbons. The alkane diol may be selected from one or more of 1,2-pentanediol, 1,2-hexanediol and 1,2-octanediol. The alkane diol may be 1,2-octanediol. It has been found that the antimicrobial activity of C5 alkane diols is surprisingly poor, the reasons for which are not fully understood (see Table 1A of the examples). Preferably, therefore, the alkane diol comprises at least 6 carbons.

The alkane diol may comprises 9 to 12 carbons, preferably 10, 11 or 12 carbons, most preferably 10 carbons. The alkane diol may be selected from 1,2-decanediol and 1,2-dodecanediol. It has been surprisingly found that alkane diols comprising 9 to 12 carbons offer greater biocidal efficacy than lower alkane diols.

Preferably, the composition further comprises a water-soluble organic solvent. Preferably, the organic solvent comprises one or more of ethylene glycol, diethylene glycol, polyethylene glycol, propylene glycol, dipropylene glycol, polypropylene glycol, phenoxyethanol and benzyl alcohol. Phenoxyethanol and benzyl alcohol have been found to be advantageous as solvents, since they also in themselves act as microbiocidal agents. .

The composition may further comprise water.

Preferably, the composition further comprises (i) propylene glycol and/or dipropylene glycol and (ii) water.

- 5 -

The composition may further comprise one or more additional microbial agents. Examples of the additional microbial agents include one or more of benzyl alcohol, 2,4-dichlorobenzyl alcohol, 2-phenoxyethanol, 2-phenoxyethanol hemiformal, phenylethyl alcohol, 5-bromo-5-nitro-1,3-dioxane, formaldehyde and formaldehyde-releasing substances, dimethylol dimethylhydantoin, glyoxal, glutaraldehyde, sorbic acid, benzoic acid, salicylic acid, d-hydroacetic acid, p-hydroxybenzoic acid ester, chloroacetamide, N-methylchloroacetamide, phenols (such as p-chloro-m-cresol and o-phenylphenol), N-methylurea, N,N'-dimethylurea, benzyl formal, 4,4-dimethyl-1,3-oxazolidine, 1,3,5-hexahydrotriazine, a quarternary ammonium compound (such as N-alkyl-N,N-dimethylbenzyl ammonium chloride and di-n-decyldimethyl ammonium chloride), cetyl pyridinium chloride, diguanidin, polyhexamethylenebiguanide, chlorhexidine, 1,2-dibromo-2,4-dicyanobutane, 3,5-dichloro-4-hydroxybenzaldehyde, ethylene glycol hemiformal, tetra-(hydroxymethyl)-phosphonium salts, dichlorophene, 2,2-dibromo-3-nitrilopropionic acid amide, methyl-N-benzimidazole-2-ylcarbamate, 2-n-octylisothiazolin-3-one, 4,5-trimethyl-2-methylisothiazolin-3-one, 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one, 2,2'-dithio-dibenzoic acid-di-N-methylamide, benzisothiazolone derivatives, 2-thiocyanomethylthiobenzothiazole, 2-(hydroxymethyl)aminoethanol, p-tolyldiiodomethylsulphone, 1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride, and a C-formal (such as 2-hydroxymethyl-2-nitro-1,3-propandiol, 2-bromo-2-nitropropane-1,3-diol, and reaction products of hydantoin). Of these, 2-phenoxyethanol and benzylalcohol, either separately or in combination, are particularly preferred.

It will be appreciated that the microbiocidal composition of the present invention may contain other ingredients. For

- 6 -

example, the composition may further comprise one or more ingredients selected from thickeners, anti-foaming agents, substances for adjusting pH values, aromas, dispersion agents and colouring agents.

The present invention further provides for the use of the microbiocidal composition as herein described in a cosmetic formulation and also a cosmetic formulation comprising the composition. The cosmetic formulation may be, for example, a moisturising cream, sunscreen, liquid soap, conditioner or shampoo.

The composition of the present invention may also be used in products such as paints, plaster compositions for the application to walls, lignin sulphonates, whitewashes, adhesives, photochemicals, products containing casein, products containing starch, asphalt emulsions, surfactant solutions, fuels, cleaning agents, water systems, polymer dispersions, and lubricants.

The microbiocidal composition according to the present invention may typically constitute 30 to 3000 parts per million of the total weight of the cosmetic formulation.

Preferably, the alkane diol is present in the composition (or in the product/cosmetic formulation) in a concentration of 500 ppm or above. At these concentrations, it has surprisingly been found that as little as 10 ppm of a 2-alkyl-3-isothiazolone can be used to prevent growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Preferably, the alkane diol is present in the composition (or in the product/cosmetic formulation) in a concentration of 1000 ppm or above. At these concentrations, it has surprising

- 7 -

been found that as little as 50 ppm of a 2-alkyl-3-isothiazolone can be used to prevent growth of *Aspergillus niger* and *Enterococcus faecalis*.

Preferably, the alkane diol is present in the composition (or in the product/cosmetic formulation) in a concentration of 2000 ppm or above, more preferably 2500 ppm or above. At these concentrations, it has surprisingly been found that as little as 25 ppm of a 2-alkyl-3-isothiazolone can be used to prevent growth of *Candida albicans*.

Preferably, the composition (or the product/cosmetic formulation) contains a maximum of 4000 ppm of the alkane diol, more preferably a maximum of 3000 ppm.

Preferably, the composition (or the product/cosmetic formulation) contains 1ppm to 300 ppm, more preferably 5ppm to 200 ppm, more preferably 10 ppm to 100 ppm, preferably 30 to 75 ppm, more preferably from 55 ppm to 75 ppm of the 2-alkyl-3-isothiazolone. Preferably the composition comprises from 1000 ppm to 3000 ppm of the alkane diol, more preferably from 1500 ppm to 2500 ppm alkane diol. The chlorphenesin is preferably present in the composition in an amount of 30 to 4000 ppm. The low concentration of the isothiazolone would not on its own be expected to prevent growth of bacteria and fungi to a satisfactory degree, if at all. However, the additional presence of the alkane diol and/or the chlorphenesin results unexpectedly in a composition with effective biocidal properties.

The composition preferably contains no chlorinated isothiazolones.

- 8 -

The composition may be made by combining and preferably mixing the components. The composition may be made at room temperature (about 20 °C) or with heating, as desired.

The cosmetic formulation according to the present invention may be in the form of a cream, a lotion, a paste, a liquid, an aerosol, a shampoo, a gel, a wipe, a bar, a stick, a powder and/or granules or any other form suitable for application to the skin, including the scalp and the mucosa including the lips.

The compositions or formulations according to the present invention preferably are formulated into forms that are useful in sunscreen products. In particular, they are preferably formulated as emulsions.

The cosmetic formulations according to the present invention may additionally contain one or more conventional ingredients or additives such as a surfactant, an emulsifier, a consistency factor, a conditioner, an emollient, a skin caring ingredient, a moisturizer, a thickener, a glidant, a lubricant, a filler, a binding agent, an anti-oxidant, a preservative, an active ingredient (eg dermatologically active ingredients) and a fragrance. Examples of active ingredients include anti-inflammatories and anti-allergics. Active ingredients suited for topical applications are particularly preferred.

Suitable surfactants include, but are not limited to, alkyl sulphates (for example, sodium lauryl sulphate, ammonium lauryl sulphate, sodium cetearyl sulphate) alkyl sulphoacetates (for example sodium lauryl sulphoacetate) alkyl ether sulphates (for example sodium laureth sulphate, sodium trideceth sulphate, sodium oleth sulphate, ammonium laureth

sulphate), alkyl ether sulposuccinates (for example disodium laureth sulposuccinate) alkyl glycosides (for example decyl glucoside, lauryl glucoside), alkyl isethionates, amphoterics (for example cocamidopropyl betaine, sodium cocoamphoacetate, sodium lauroamphoacetate, disodium lauroamphodiacetate, disodium cocoamphodiacetate, sodium lauroamphopropionate, disodium lauroamphodipropionate, potassium or ammonium salts of the aforementioned amphoterics, capryl/capramidopropyl betaine, undecylenamidopropyl betaine, lauramidopropyl betaine) and fatty alcohol polyglycol ethers.

Suitable emulsifiers include, but are not limited to, salts of fatty acids (for example sodium stearate or sodium palmitate), organic soaps (for example, mono-, di- or triethanolaminoleate), sulphates or sulphonated compounds (for example sodium lauryl sulphate or sodium cetyl sulphonate), saponines, lamepones, quaternary ammonium salts, fatty alcohols, fatty acid esters derived from saturated or unsaturated fatty acids, polyoxyethylenesters or polyoxyethylenethers of fatty acids, polymers of ethylene oxide and propylene oxide or propylene glycol, phosphatides, gelatine, casein alkylamidobetaines, alkyl betaines, alkyl amphoglycinates, alkyl phosphates, alkylpolyoxyethylene phosphates or the corresponding acids and silicone derivatives (for example alkyl dimethiconecopolyol).

Suitable consistency factors include, but are not limited to, fatty alcohols or their mixtures with fatty acid esters, (for example, acetylated lanolin alcohol), aluminium stearates, carbomer, cetyl alcohol, glyceryl oleate, glyceryl stearate, glyceryl stearate, PEG 100 stearate, magnesium stearate, magnesium sulphate, oleic acid, stearic acid, stearyl alcohol, myristyl myristate, isopropyl palmitate and beeswax and synthetic equivalents thereof.

- 10 -

Suitable conditioners include, but are not limited to, alkylamido ammonium lactate, cetrimonium chloride, distearoylethyl hydroxyethylammonium methosulphate, cetearyl alcohol, cetyl dimethicone, cetyl ricinoleate, dimethicone, laureth-23, laureth-4, polydecene, retinyl palmitate, quaternised protein hydrolysates, quaternised cellulose and starch derivatives, quaternised copolymers of acrylic or methacrylic acid or salts and quaternised silicone derivatives.

Suitable emollients include, but are not limited to, cetearyl isononanoate, cetearyl octanoate, decyl oleate, iso-octyl stearate, coco caprylate/caprate, ethylhexyl hydroxystearate, ethylhexyl isononanoate, isopropyl isostearate, isopropyl myristate, oleyl oleate, hexyl laurate, paraffinum liquidum, PEG-75 lanolin, PEG-7 glyceryl cocoate, petrolatum, ozokerite, cyclomethicone, dimethicone, dimethicone copolyol, dicaprylyl ether, butyrospermum parkii, buxus chinensis, canola, carnauba cera, copernicia cerifera, oenothera biennis, elaeis guineensis, prunus dulcis, squalane, zea mays, glycine soja, lanolin, hydrogenated castor oil, hydrogenated coconut oil, hydrogenated polyisobutene, sucrose cocoate, stearoxy dimethicone, lanolin alcohol and isohexadecane.

Suitable skin caring ingredients include, but are not limited to, plant extracts, bisabolol, anti-inflammatory agents, urea, allantoin, panthenol and panthenol derivatives, phytantriol, vitamins A, B5, E, C and D, ceramides of animal or plant origin and lecithins.

Suitable moisturizers include, but are not limited to, butylene glycol, cetyl alcohol, dimethicone, dimyristyl

- 11 -

tartrate, glucose, glycereth-26, glycerin, glyceryl stearate, hydrolyzed milk protein, lactic acid, lactose and other sugars, laureth-8, lecithin, octoxyglycerin, PEG-12, PEG-135, PEG-150, PEG-20, PEG-8, phytantriol, polyquaternium-39, PPG-20 methyl glucose ether, propylene glycol, sodium hyaluronate, sodium lactate, sodium PCA, sorbitol, succinoglycan, synthetic beeswax, tri-C₁₄₋₁₅ alkyl citrate and starch.

Suitable thickeners include, but are not limited to, acrylate/steareth-20 methacrylate copolymers, carbomer, carboxymethyl starch, cera alba, dimethicone/vinyl dimethicone crosspolymer, propylene glycol alginate, hydroxyethylcellulose, hydroxypropyl methylcellulose, silica, silica dimethyl silylate, xanthan gum and hydrogenated butylenes/ethylene/styrene copolymer.

Suitable lubricants include, but are not limited to, adipic acid, fumaric acid and its salts, benzoic acid and its salts, glycerine triacetate, sodium or magnesium lauryl sulphate, magnesium stearate, solid polyethylenglycol, polyvinylpyrrolidone, boric acid, monolaurate or - palmitate, myristyl alcohol, cetyl alcohol, cetylstearyl alcohol, talcum, calcium or magnesium salts of higher fatty acids, mono-, di- or triglycerides of higher fatty acids and polytetrafluorethylene.

Suitable anti-oxidants include, but are not limited to, sulphites (for example, sodium sulphite, tocopherol or derivates thereof), ascorbic acid or derivates thereof, citric acid, propyl gallate, chitosan glycolate, cysteine, N-acetyl cysteine plus zinc sulphate, thiosulphates (for example sodium thiosulphate) and polyphenoles.

Examples

The following Examples (see Tables I to VI) show the unexpected synergy of the non-halogenated 2-alkyl-3-isothiazolone and the alkane diol in the preservative composition of the invention.

For this purpose, aqueous mixtures were produced with various concentrations of 2-methylisothiazolin-3-one (MIT) and caprylyl glycol (CAG) and the activity of these mixtures were tested on the micro-organisms listed in Table VI.

In addition to the active component(s) and water, the aqueous mixtures contained a nutrient medium, namely a Müller-Hinton broth (Manufacturer: Oxoid; product code CM405). The incubation time was 72h at 25°C (bacterial and yeast) or 7 days at 25°C (mould). Each sample was incubated on an incubation shaker at 105 rpm.

Tables I to V show the concentrations of MIT and CAG used in each Example. The tables also show whether growth of the micro-organism took place (symbol "+") or not (symbol "-").

Tables I to V also show the minimum inhibition concentrations (MIC). For example, in Table I, an MIC value of 25 ppm is found when MIT is used alone and an MIC value of 4000 ppm when CAG is used alone. In contrast, the MIC value of a mixture of MIT and CAG is clearly lower, i.e., when they are combined, MIT and CAG act synergistically.

- 13 -

Table I

MIC values for *Pseudomonas aeruginosa* after an incubation time of 72h

MIT	CAG concentration (ppm)					
concentration (ppm)	0	500	1000	2000	3000	4000
75	-	-	-	-	-	-
50	-	-	-	-	-	-
37.5	-	-	-	-	-	-
25	-	-	-	-	-	-
10	+	-	-	-	-	-
0	+	+	+	+	+	-

Table II

MIC values for *Staphylococcus aureus* after an incubation time of 72h

MIT concentration (ppm)	CAG concentration (ppm)					
	0	500	1000	2000	3000	4000
75	-	-	-	-	-	-
50	-	-	-	-	-	-
37.5	-	-	-	-	-	-
25	-	-	-	-	-	-
10	+	-	-	-	-	-
0	+	+	-	-	-	-

Table III

- 14 -

MIC values for *Enterococcus faecalis* after an incubation time of 72h

MIT concentration (ppm)	CAG concentration (ppm)					
	0	500	1000	2000	3000	4000
75	-	-	-	-	-	-
50	+	-	-	-	-	-
37.5	+	+	+	-	-	-
25	+	+	+	-	-	-
10	+	+	+	-	-	-
0	+	+	+	-	-	-

Table IV

MIC values for *Aspergillus niger* after an incubation time of 5 days

MIT concentration (ppm)	CAG concentration (ppm)					
	0	500	1000	2000	3000	4000
300	-	-	-	-	-	-
200	+	-	-	-	-	-
100	+	+	-	-	-	-
75	+	+	-	-	-	-
50	+	+	-	-	-	-
37.5	+	+	+	-	-	-
25	+	+	+	-	-	-
10	+	+	+	-	-	-
0	+	+	+	-	-	-

Table V

MIC values for *Candida albicans* after an incubation time of 72h

MIT concentration (ppm)	CAG concentration (ppm)					
	0	500	1000	2000	3000	4000
200	-	-	-	-	-	-
100	+	-	-	-	-	-
75	+	-	-	-	-	-
50	+	+	-	-	-	-
37.5	+	+	+	-	-	-
25	+	+	+	-	-	-
10	+	+	+	+	-	-
0	+	+	+	+	-	-

The synergy index is calculated according to the method of F.C. Kull et al., Applied Microbiology, Vol. 9 (1961), p. 538. Here the synergy index is calculated using the following formula:

$$\text{Synergy index} = Q_a/Q_A + Q_b/Q_B$$

When this formula is used on the biocide system tested here, the quantities in the formula have the following meaning:

Q_a = MIC of MIT in the preservative mixture of MIT and CAG

Q_A = MIC of MIT as sole ingredient

Q_b = MIC of CAG in the preservative mixture of MIT and CAG

Q_B = MIC of CAG as sole ingredient

- 16 -

When the synergy index exhibits a value greater than 1, this means that there is an antagonism. When the synergy index has the value 1, this means that there is an addition of the activity of the two ingredients. When the synergy index has a value of less than 1, this means that a synergy of the two ingredients exists.

Table VI
Synergy indices calculated from data in Tables I - V

Organism	Strain	MIC values (ppm)				Synergy index
		Q _A (MIT)	Q _B (CAG)	Q _a (MIT)	Q _b (CAG)	
<i>Pseudomonas aeruginosa</i>	ATCC 9027	25	4000	10	500	0.53
<i>Staphylococcus aureus</i>	ATCC 6538	25	1000	10	500	0.90
<i>Enterococcus faecalis</i>	NCIMB 12756	75	2000	50	500	0.92
<i>Aspergillus niger</i>	IMI 149007	300	2000	50	1000	0.67
<i>Candida albicans</i>	NCPF 3179	200	3000	75	500	0.55

Test on Synergistic effect of Chlorphenesin

A test was also carried out to assess if chlorphenesin exhibited a synergistic effect when combined with a composition comprising both MIT and CAG. The test was carried out in a similar manner as above.

For this purpose, aqueous mixtures were produced containing various concentrations of both (i) a composition comprising MIT and CAG in a 1:30 weight ratio and (ii) chlorphenesin and the activity of these mixtures were tested on the micro-organisms listed in Table VII.

- 17 -

In addition to the active component(s) and water, the aqueous mixtures contained a nutrient medium, namely a Müller-Hinton broth (Manufacturer: Oxoid; product code CM405). The incubation time was 72h at 25°C (bacterial and yeast) or 7 days at 25°C (mould). Each sample was incubated on an incubation shaker at 105 rpm.

In an analogous manner as for the Examples above, for each micro-organism, the MIC was determined for a composition comprising MIT and CAG in a weight ratio of 1:30 (i) in the absence of chlorphenesin ($Q_{A(MIC+CAG)}$) and (ii) in the presence of chlorphenesin ($Q_{a(MIC+CAG)}$).

The MIC of chlorphenesin was likewise determined (i) for chlorphenesin as a sole ingredient ($Q_{B(CP)}$) and (ii) for chlorphenesin in the presence of a composition comprising MIT and CAG in a weight ratio of 1:30 ($Q_{b(CP)}$).

The synergy index (SI) for each micro-organism was calculated as follows:

$$\text{Synergy index} = Q_{a(MIC+CAG)} / Q_{A(MIC+CAG)} + Q_{b(CP)} / Q_{B(CP)}.$$

The results are as shown in Table VII:

Table VII

	$Q_A(\text{MIT/CAG})$	$Q_B(\text{CP})$	$Q_a(\text{MIT/CAG})$	$Q_b(\text{CP})$	SI
<i>E. Coli</i>	620	1500	310	200	0.70
<i>Staphylococcus aureus</i>	620	3000	310	1000	0.83
<i>Candida albicans</i>	930	2000	620	400	0.87

- 18 -

One can see from this table that chlorphenesin and a composition of MIT and CAG acted synergistically for *E. Coli*, *St. aureus* and *Candida albicans*.

Analogous experiments were carried out as above for C₁₀ to C₁₂ alkane diols. It was surprising found that C₁₀ to C₁₂ alkane diols exhibited greater biocidal efficacy than C₈ alkane diols when in combination with MIT.

Tables VIIIA to VIIID - Efficacy of C₅ alkane diols vs. C₈ alkane diols.

Table VIIIA. *Pseudomonas aeruginosa*

	0%	0.2%	0.4%	0.6%	0.8%	1.0%
1,2 Pentane diol	+	+	+	+	+	+
1,2-Octane diol	+	+	-	-	-	-

Table VIIIB. *Staphylococcus Aureus*

	0%	0.2%	0.4%	0.6%	0.8%	1.0%
1,2 Pentane diol	+	+	+	+	+	+
1,2-Octane diol	+	-	-	-	-	-

Table VIIIC. *Candida Albicans*

	0%	0.2%	0.4%	0.6%	0.8%	1.0%
1,2 Pentane diol	+	+	+	+	+	+
1,2-Octane diol	+	+	-	-	-	-

Table VIIID. *Aspergillus Niger*

	0%	0.2%	0.4%	0.6%	0.8%	1.0%
1,2 Pentane diol	+	+	+	+	+	+
1,2-Octane diol	+	-	-	-	-	-

+ = no growth inhibition

- = growth inhibition

It has been found by the inventors that alkane diols exhibit different anti-microbial activities. Tables VIIIA to VIIID above illustrate the results of tests of the efficacy of octane diol compared to pentane diol on various organisms. These tests were carried out in a similar manner to those described above. The percentages at the top line of each table indicate the amount of diol used by weight in the preservative mixture.

Test on a model formulated skin cream

Preservative Efficacy Tests (PETs)

Typical formulations of the active components were subjected to a PET using a model formulated skin cream of the following composition:

Skin Cream

Material	INCI	%
Water Deionised	Aqua to	100
Polawax GP200		8.0
Glycerine BP	Glycerin	5.0
Propylene Glycol BP	Propylene Glycol	5.0
Fractionated Coconut Oil	Caprylic/Capric Triglyceride	4.0

Sweet Almond Oil	Prunus Dulcis	4.0
Light Liquid Paraffinum	Paraffinum Liquidum	3.0
RD Coconut Oil	Cocus Nucifera Oil	2.0
Lasermul 92AE	Glyceryl Stearate SE	2.0
Aloe Vera x 10	Aloe Barbadensis	1.0
Crodamol SS	Cetyl Esters	1.0
Cucumber Extract	Cucumis Sativus	0.5
Ground Ivy Extract	Glechoma Hederacea	0.5
Ginseng Extract	Panax Ginseng	0.5
Fragrance	Parfum	0.5
Vitamin E Acetate	Tocopheryl Acetate	0.2
Vitamin A Palmitate	Retinyl Palmitate	0.1
FD&C Blue No 1	CI 42090	0.0003
FD&C Yellow No 5	CI 19140	0.0005

The test procedure, based on the British Pharmacopoeia 2004 (Appendix XVIC), was as follows. Aliquots of the preserved skin cream were inoculated using single bacteria or fungi. The inoculated aliquots were incubated at 25°C and Ig samples removed after 0, 2, 7, 14 and 28 days. Total viable counts (TVC) to determine surviving bacteria or fungi were performed after preservative inactivation using Letheen Broth. Where applicable serial dilutions were carried out using quarter strength Ringers Solution. Bacteria were plated onto Tryptone Soya Agar and incubated at 33°C for 72 hours, whilst fungi were plated onto Sabouraud Dextrose Agar and incubated at 23°C for 120 hours.

The pass criteria for the preservatives were as follows:

- 21 -

Bacteria

Reduction in TVC of 10^2 bacteria (with respect to NC at Time 0) within 48 hours of the challenge and 10^3 bacteria after 7 days with no increase thereafter.

Table IX PET Results for Microcare® MTG (Mixture of MIT and CAG)

Organism	Preservative %	0 days	2 days	7 days	14 days	28 days
A. niger IMI 149007	0.20	2.0×10^5	1.8×10^5	6.0×10^4	4.0×10^4	1.0×10^4
	0.30	1.6×10^5	6.2×10^4	1.2×10^4	2.0×10^4	1.6×10^3
	0.40	2.2×10^5	1.6×10^4	4.0×10^3	1.0×10^3	2.0×10^1
	0.50	2.6×10^5	2.6×10^3	2.0×10^2	<20	<20
C. albicans NCPF 3197	0.20	1.0×10^6	8.0×10^5	1.0×10^5	2.0×10^5	1.2×10^5
	0.30	4.0×10^5	1.2×10^5	2.0×10^2	<20	<20
	0.40	1.0×10^6	6.0×10^5	<20	<20	<20
	0.50	4.0×10^5	1.0×10^5	<20	<20	<20
Ps. Aeruginosa ATCC 9027	0.20	4.0×10^6	5.0×10^5	<20	<20	<20
	0.30	4.0×10^6	2.6×10^5	<20	<20	<20
	0.40	1.8×10^7	1.1×10^5	<20	<20	<20
	0.50	1.2×10^7	2.4×10^4	<20	<20	<20
S. aureus ATCC 6538	0.20	3.2×10^7	1.0×10^7	<20	<20	<20
	0.30	2.8×10^7	9.4×10^6	<20	<20	<20
	0.40	4.8×10^7	7.2×10^6	<20	<20	<20
	0.50	5.6×10^7	1.6×10^6	<20	<20	<20
B. cepacia NCIMB 9085	0.20	8.0×10^6	1.8×10^6	<20	<20	<20
	0.30	4.0×10^6	6.2×10^5	<20	<20	<20
	0.40	1.0×10^7	6.6×10^4	<20	<20	<20
	0.50	1.4×10^7	5.0×10^3	<20	<20	<20

-23-

Table X PET Results for Microcare® MTC (Mixture of MIT and Chlorphenesin)

Organism	WR218 (%)	0 hours	48 hours	7 days	14 days	28 days
A. niger IMI 14907	0.20	4.0×10^5	2.0×10^5	2.0×10^5	2.0×10^5	6.0×10^4
	0.60	4.0×10^5	4.0×10^5	8.0×10^4	2.0×10^4	2.0×10^4
	1.00	2.0×10^5	6.0×10^4	4.0×10^4	4.0×10^2	<20
C. albicans NCPF 3179	0.20	5.0×10^5	1.3×10^6	2.0×10^6	8.6×10^5	6.0×10^5
	0.60	7.4×10^5	2.6×10^5	4.4×10^4	2.0×10^4	4.0×10^2
	1.00	6.6×10^5	2.6×10^5	2.0×10^2	<20	<20
Ps. aeruginosa ATCC 9027	0.20	1.4×10^7	2.2×10^6	<20	<20	<20
	0.60	8.0×10^6	5.2×10^5	<20	<20	<20
	1.00	6.2×10^6	1.6×10^5	<20	<20	<20
S. aureus ATCC 6538	0.20	1.8×10^7	1.8×10^7	2.2×10^5	<20	<20
	0.60	3.0×10^7	2.6×10^7	2.8×10^5	<20	<20
	1.00	1.6×10^7	1.2×10^7	4.6×10^4	<20	<20
B. cepacia NCIMB 9085	0.20	6.2×10^6	8.0×10^5	1.6×10^4	<20	<20
	0.60	6.2×10^6	1.2×10^6	2.0×10^1	<20	<20
	1.00	6.2×10^6	2.0×10^6	<20	<20	<20

Claims:

1. A microbiocidal composition comprising:
a non-halogenated 2-alkyl-3-isothiazolone, and
an alkane diol and/or chlorphenesin,
wherein the alkyl group in the non-halogenated 2-alkyl-3-isothiazolone comprises 1 to 4 carbon atoms,
the alkane diol comprises from 5 to 12 carbon atoms, and
the hydroxyl groups of the alkane diol are vicinally substituted.
2. A microbiocidal composition according to claim 1, wherein the alkane diol comprises from 9 to 12 carbon atoms.
3. A microbiocidal composition according to claim 2, wherein the alkane diol comprises 10 carbon atoms.
4. A microbiocidal composition according to claim 1, wherein the alkane diol comprises 6 to 8 carbon atoms.
5. A microbiocidal composition according to claim 4, wherein the alkane diol is 1,2-octanediol.
6. A microbiocidal composition according any one of the preceding claims comprising the 2-alkyl-3-isothiazolone and the alkane diol in a ratio range (wt.%) of from 1:10 to 1:30.
7. A microbiocidal composition according to any one of the preceding claims comprising the 2-alkyl-3-isothiazolone and the alkane diol in a ratio range (wt.%) of from 1:10 to 1:20.
8. A microbiocidal composition according to claim 1 comprising the 2-alkyl-3-isothiazolone and the chlorphenesin in a ratio range (wt.%) of from 1:10 to 1:30.

9. A microbiocidal composition according to any one of the preceding claims comprising the non-halogenated 2-alkyl-3-isothiazolone, the alkane diol and the chlorphenesin.
10. A microbiocidal composition according to any one of the preceding claims, wherein the non-halogenated 2-alkyl-3-isothiazolone is 2-methyl-3-isothiazolone.
11. A microbiocidal composition according to any one of the preceding claims, wherein the composition further comprises a water-soluble organic solvent.
12. A microbiocidal composition according to claim 11, wherein the organic solvent comprises one or more of ethylene glycol, diethylene glycol, polyethylene glycol, propylene glycol, dipropylene glycol and polypropylene glycol.
13. A cosmetic formulation comprising a microbiocidal composition as defined in any one of claims 1 to 12.
14. A cosmetic formulation as claimed in claim 13, wherein the formulation contains from 1 ppm to 100 ppm of the 2-alkyl-3-isothiazolone, and from 300 ppm to 4000 ppm of the alkane diol and/or from 30 to 4000 ppm chlorphenesin.
15. A cosmetic formulation according to claim 13 or claim 14, wherein the cosmetic formulation is selected from a moisturising cream, a sunscreen, a liquid soap and a shampoo.
16. Use of the microbiocidal composition according to any one of claims 1 to 12 in a cosmetic formulation.

- 26 -

17. Use of the microbiocidal composition according to claim 16 in moisturising cream, sunscreen, liquid soap, conditioner or shampoo.

18. Use of the microbiocidal composition as defined in any one of claims 1 to 12 in product selected from: a paint, a plaster composition, a lignin sulphonate, a whitewash, an adhesive, a photochemical composition, a product containing casein, a product containing starch, an asphalt emulsion, a surfactant solution, a fuel, a cleaning agent, a water system, a polymer dispersion or a lubricant.

19. Use according to claim 18, wherein the product contains from 1 ppm to 300 ppm of the 2-alkyl-3-isothiazolone, and from 300 ppm to 4000 ppm of the alkane diol and/or from 30 to 4000 ppm chlorphenesin.

A. CLASSIFICATION OF SUBJECT MATTER

INV. A01N43/80 A61K8/49 A61Q17/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	US 5 306 725 A (Y. SANO ET AL.) 26 April 1994 (1994-04-26) * Claim 1 ; comparative example 9 ; column 1, line 18-24 *	1-19
X	US 4 824 957 A (D.R. AMICK) 25 April 1989 (1989-04-25) column 3, line 31 - line 51; claim 1; examples 1,3	1-19
X	US 6 447 793 B2 (D. AUST ET AL.) 10 September 2002 (2002-09-10) column 2, line 39 - line 48; claim 1	1-19
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☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents :

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INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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International application No

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