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(54) **Title:** METHOD AND COMPOSITIONS FOR IMPROVING SELECTIVE CATABOLYSIS AND VIABILITY IN CELLS OF KERATIN SURFACES

(57) **Abstract:** A composition for treating keratin surfaces to stimulate selective catabolysis and improve cellular viability comprising at least one autophagy activator and at least one DNA repair enzyme, and a method for improving selective catabolysis and cellular viability by treating with the composition.



DNA repair enzymes are known to repair DNA in various ways. Such enzymes are generally categorized according to the type of DNA base mutagenic damage that is repaired.

It has been discovered that the combination of DNA repair enzymes and autophagy activators most unexpectedly will beneficially affect the normal cellular cleansing processes, promote cellular health and longevity, and minimize the deleterious effects of environment, UV light, pollution, and other environmental insults on skin.

Accordingly it is of interest to maximize the cellular health and longevity of cells, particularly skin cells such as keratinocytes or fibroblasts, by treating them with ingredients that stimulate natural cellular repair processes by, for example, eliminating cellular debris and toxins, and in addition, to maximize the effectiveness of such cellular detoxification by ensuring that the maximum number of detoxification mechanisms are operable. It is also of interest to stimulate detoxification processes where the breakdown of the cellular debris and toxins results in amino acids or other biological molecules that can be recycled by the cell.

Thus, there is a need to maximize cellular health and longevity by stimulating selective catabolysis in cells so that cellular debris and toxins are removed and by products of such degradation may be recycled. More preferably, the selective catabolysis is due to enhancing autophagy activity and promoting DNA repair using DNA repair enzymes.

It has been discovered that compositions containing at least one autophagy activator and at least one DNA repair enzyme in selective cellular catabolysis, that is, cleansing cells of toxic waste products by breaking down the waste products into molecules that may be recycled and used by the cell in normal metabolic functions.

### Summary of the Invention

The invention is directed to a composition for treatment of keratin surfaces to stimulate selective catabolysis comprising at least one autophagy activator and at least one DNA repair enzyme.

The invention is further directed to a method for stimulating selective catabolysis in cells of keratin surfaces by applying to such surfaces a composition comprising at least one autophagy activator and at least one DNA repair enzyme.

Detailed DescriptionI. Definitions

All percentages mentioned herein are percentages by weight unless otherwise  
5 indicated.

All documents mentioned herein are incorporated by reference in their entirety.

“Autophagy” means the process by which cells cleanse themselves of toxins and debris  
by forming a membrane around the debris, segregating it from the rest of the cell, and  
10 adjoining the formed vacuole with cellular lysosomes, which are cellular organelles that  
contain acid hydrolase enzymes that break down the cellular debris and waste found in the  
vacuole.

“Autophagy Activator” means an ingredient that stimulates the normal cellular  
15 autophagy processes.

“CLOCK gene activator” means an ingredient that activates one or more CLOCK genes  
present in keratinocytes.

20 The term “DNA repair enzyme” means an enzyme that is operable to repair DNA base  
mutagenic damage. Such enzymes are often categorized by the type of DNA damage they  
repair, for example BER (base excision repair) enzymes, nucleotide excision repair (NER)  
enzymes; mismatch repair (MMR) enzymes; DNA helicases; DNA polymerases, and so on.  
For example, mutations such as 8-oxo-7,8-dihydro-2'-deoxyguanosine may be repaired by  
25 OGG1 (8-oxoGuanine glycosylase); T-T dimers which may be repaired by (Nucleotide  
excision repair (NER) Photolyase); 6-4 photoproducts (which may be repaired by NER); and  
06-methyl guanine (which may be repaired by 06-alkyl guanine transferase (AGT)).

The term “keratin surfaces” means skin, hair, and nails.

30 “PER1 gene activator” means an ingredient that activates one or more PER1 genes  
found in keratinocytes.

“Proteasome” means a protein complex typically located in the nucleus or cytoplasm of cells that is operable to degrade damaged cellular proteins by proteolysis into smaller subunits which may then be further digested into single amino acids. These recycled amino acids may be used by the cell in the synthesis of new proteins.

5

“Proteasome activator” means an active ingredient that stimulates the activity of proteasomes in cells of keratin surfaces such as keratinocytes, fibroblasts, etc.

“Recycle” means, with respect to the degradation of cellular debris and toxins, that the debris and toxins may be broken down into molecules such as proteins, lipids, amino acids, or other biological materials that are usable by the cell in its normal healthy metabolic processes.

10

“Repair” means, with respect to skin cells, that the damaged portions of cells, such as DNA, mitochondria, proteins, lipids, or other cellular materials are reduced or eliminated.

15

“Selective catabolysis” means, with respect to the cells of keratin surfaces, that the cells are able to cleanse themselves of debris, waste, and toxins selectively without compromising healthy cellular constituents, and preferably by one or more of mechanisms such as activating cellular autophagy or activating cellular proteasome processes.

20

## II. Autophagy Activator

25

The composition of the invention contains at least one ingredient that is operable to activate normal cellular autophagic processes. The autophagy activator is present in amounts ranging from about 0.00001 to 20%, preferably 0.0001-15%, more preferably from about 0.001 to 10%. In general, the cellular autophagy process comprises four general steps. Step 1 is the initiation of vacuole formation; Step 2 the formation of the initial vacuole or autophagosome which sequesters the cytoplasmic material to be degraded. Step 3 is the maturation of the autophagosome into a degradative vacuole. Step 4 is the actual degradation of the sequestered material.

30

Ingredients with autophagy activation activity can be identified by their ability to either stimulate or inhibit various cellular metabolic pathways. For example, ingredients that

stimulate the expression of MAP-LC3, ATG5-12, protein p53, AMPK, or DRAM are suitable autophagy activators. Ingredients that inhibit the expression of mTOR are also suitable autophagy activators.

5           The gene MAP-LC3 codes for microtubule-associated protein 1 light chain 3, a protein that initiates formation of autophagosomes. ATG5-12 also stimulates formation of autophagosomes. mTOR, also known as mammalian target of rapamycin, is also known as the mechanistic target of rapamycin or FK506 binding protein 12-rapamycin associated protein 1 (FRAP1). FRAP1 is encoded by the FRAP gene. Any ingredient that inhibits the expression  
10 of mTOR, involved in autophagosome creation, will have autophagy activating properties. Also suitable as autophagy activators are ingredients that stimulate expression of protein p53, AMPK, and/or DRAM (damage remedy autophagy modulator protein) in keratinocytes. Protein p53, also known as a tumor suppressor protein, is encoded by the p53 gene. AMPK means AMP activated protein kinase and DRAM, damage related autophagy modulator. Both  
15 are known to stimulate autophagy activation in keratinocytes.

          Thus any ingredient that has the above mentioned effects on the genes may be suitable autophagy activators. During the autophagocytic process cellular debris such as oxidized proteins and peroxidized lipids are degraded. Such cellular debris often affects normal  
20 metabolic function. Screening of ingredients to determine efficacy by ability to stimulate or inhibit cellular, preferably keratinocyte, genes and/or proteins mentioned above may be done according to methods as set forth in US Patent Publication No. 2011/0243983 or other methods known in the art.

25           For example, one general process for identifying ingredients that may be autophagy activators is by first inducing nutritive stress in cultured cells such as keratinocytes. For example, the cells are first cultured in complete culture medium with growth factors, for about 24 hours. The culture medium is then removed and replaced with a non-nutritive culture medium, for example one that does not contain growth factors. The cells are cultured for  
30 about 30 minutes to about 25 hours in a state of nutritive stress. Then, the non-nutritive culture medium is removed and replaced with complete culture medium to promote cellular recovery. Thereafter, the cells are evaluated for autophagocytic activity by measuring the expression of one or more of MAP-LC3; ATGS-12; phosphorylated mTOR; phosphorylated p53; DRAM; or phosphorylated AMPK in those cells. Measurement of such expression can

take place by immunofluorescence measurements. In addition, the expression can be ascertained by Western Blot analysis of phosphorylated proteins associated with the expressed genes.

5           Examples of ingredients that are known to exert either the stimulatory or inhibitory effects on the above mentioned genes which, in turn, stimulate autophagy, are yeast extracts including but not limited to those from the genres such as *Lithothamnium*, *Melilot*, *Citrus*, *Candida*, *Lens*, *Urtica*, *Carambola*, *Momordica*, *Yarrowia*, *Plumbago*, etc. Further specific examples include *Lithothamnium calcareum*, *Melilotus officinalis*, *Citrus limonum*, *Candida*  
10 *saitoana*, *Lens culinaria*, *Urtica dioica*, *Averrhoa carambola*, *Momordica charantia*, *Yarrowia lipolytica*, *Plumbago zeylanica* and so on.

Also suitable are ingredients such as amiodarone hydrochloride, GF 109203X which is also referred to as (3-(N-[Dimethylamino]propyl-3-indolyl)-4-(3-indolyl)maleimide 3-[1-[3-  
15 (Dimethylamino)propyl]1H-indol-3-yl]-4-(1Hindol-3-yl)1H-pyrrole-2,5dione Bisindolylmaleimide I; N-Hexanoyl-D-sphingosine; Niclosamide; Rapamycin from *Streptomyces hygroscopicus*; Rottlerin which is also referred to as (1-[6-[(3-Acetyl-2,4,6-trihydroxy-5-methylphenyl)methyl]-5,7-dihydroxy-2,2-dimethyl-2H-1-benzopyran-8-yl]-3-phenyl-2-propen-1-one, Mallotoxin); STF-62247, also known as 5-Pyridin-4-yl-thiazol-2-yl-  
20 m-tolyl-amine; Tamoxifen; Temsirolimus which is also known as 42-[3-Hydroxy-2-methylpropanoate, CCI-779, Rapamycin; ATG1 autophagy related 1 homolog; ATG1, Serine/threonine-protein kinase ULK1, UNC-51-like kinase; or Z36 which is also referred to as ((Z)-5-Fluoro-1-(3'-dimethylamino)propyl-3-[(5'-methoxyindol-3-ylidene)methyl]-indolin-2-one; or 1-[3-(dimethylamino)propyl]-5-fluoro-1,3-dihydro-3-[(5-methoxy-1H-indol-3-yl)methylene]-2H-Indol-2-one); Bufalin, also referred to as 3 $\beta$ ,14-Dihydroxy-5 $\beta$ ,20(22)-  
25 bufadienolide, 5 $\beta$ ,20(22)-Bufadienolide-3 $\beta$ ,14-diol. Such ingredients may be purchased from Sigma-Aldrich Chemical Company.

### III. DNA Repair Enzyme

30           The composition contains one or more DNA repair enzymes. Suggested ranges are from about 0.00001 to about 35%, preferably from about 0.00005 to about 30%, more preferably from about 0.0001 to about 25% of one or more DNA repair enzymes.

DNA repair enzymes as disclosed in U.S. Patent Nos. 5,077,211; 5,190,762; 5,272,079; and 5,296,231, all of which are hereby incorporated by reference in their entirety, are suitable for use in the compositions and method of the invention. One example of such a DNA repair enzyme may be purchased from AGI/Dermatics under the trade name

5 Roxisomes®, and has the INCI name *Arabidopsis Thaliana* extract. It may be present alone or in admixture with lecithin and water. This DNA repair enzyme is known to be effective in repairing 8-oxo-Guanine base damage.

10 Another type of DNA repair enzyme that may be used is one that is known to be effective in repairing O<sup>6</sup>-methyl guanine base damage. It is sold by AGI/Dermatics under the tradename Adasomes®, and has the INCI name Lactobacillus ferment, which may be added to the composition of the invention by itself or in admixture with lecithin and water.

15 Another type of DNA repair enzyme that may be used is one that is known to be effective in repairing T-T dimers. The enzymes are present in mixtures of biological or botanical materials. Examples of such ingredients are sold by AGI/Dermatics under the tradenames Ultrasomes® or Photosomes®. Ultrasomes® comprises a mixture of *Micrococcus* lysate (an end product of the controlled lysis of various species of micrococcus), lecithin, and water. Photosomes® comprise a mixture of plankton extract (which is the extract of marine  
20 biomass which includes one or more of the following organisms: thalassoplankton, green micro-algae, diatoms, greenish-blue and nitrogen-fixing seaweed), water, and lecithin.

25 Another type of DNA repair enzyme may be a component of various inactivated bacterial lysates such as *Bifida* lysate or *Bifida* ferment lysate, the latter a lysate from *Bifido* bacteria which contains the metabolic products and cytoplasmic fractions when *Bifido* bacteria are cultured, inactivated and then disintegrated. This material has the INCI name *Bifida* Ferment Lysate.

30 Other suitable DNA repair enzymes include Endonuclease V, which may be produced by the denV gene of the bacteriophage T4. Also suitable are T4 endonuclease; O<sup>6</sup>-methylguanine-DNA methyltransferases; photolyases such as uracil- and hypoxanthine-DNA glycosylases; apyrimidinic/apurinic endonucleases; DNA exonucleases, damaged-bases glycosylases (e.g., 3-methyladenine-DNA glycosylase); correndonucleases either alone or in

complexes (e.g., *E. coli* uvrA/uvrB/uvrC endonuclease complex); APEX nuclease, which is a multi-functional DNA repair enzyme often referred to as "APE"; dihydrofolate reductase; terminal transferase; topoisomerase; O<sup>6</sup> benzyl guanine; DNA glycosylases.

5 Other types of suitable DNA repair enzymes may be categorized by the type of repair facilitated and include BER (base excision repair) or BER factor enzymes such as uracil-DNA glycosylase (UNG); single strand selective monofunctional uracil DNA glycosylase (SMUG1); 3,N(4)-ethenocytosine glycosylase (MBD4); thymine DNA-glycosylase (TDG); A/G-specific adenine DNA glycosylase (MUTYH); 8-oxoguanine DNA glycosylase (OGG1);  
10 endonuclease III-like (NTHL1); 3-methyladenine DNA glycosidase (MPG); DNA glycosylase/AP lyase (NEIL1 or 2); AP endonuclease (APEX 1 and 2), DNA ligase (LIG3), ligase accessory factor (XRCC1); DNA 5'-kinase/3'-phosphatase (PNKP); ADP-ribosyltransferase (PARP1 or 2).

15 Another category of DNA repair enzymes includes those that are believed to directly reverse damage such as O<sup>6</sup>-MeG alkyl transferase (MGMT); 1-meA dioxygenase (ALKBH2 or ALKBH3).

Yet another category of enzymes operable to repair DNA/protein crosslinks includes  
20 Tyr-DNA phosphodiesterase (TDP1).

Also suitable are MMR (mismatch excision repair) DNA repair enzymes such as MutS protein homolog (MSH2); mismatch repair protein (MSH3); mutS homolog 4 (MSH4); MutS homolog 5 (MSH5); or G/T mismatch-binding protein (MSH6); DNA mismatch repair protein  
25 (PMS1, PMS2, MLH1, MLH3); Postmeiotic segregation increased 2-like protein (PMS2L3); or postmeiotic segregation increased 2-like 4 pseudogene (PMS2L4).

Also suitable are DNA repair enzymes are those known as nucleotide excision repair (NER) enzymes and include those such as Xeroderma pigmentosum group C-complementing  
30 protein (XPC); RAD23 (*S. cerevisiae*) homolog (RAD23B); caltractin isoform (CETN2); RFA Protein 1, 2, of 3 (RPA1, 2, or 3); 3' to 5' DNA helicase (ERCC3); 5' to 3' DNA helicase (ERCC2); basic transcription factor (GTF2H1, GTF2H2, GTF2H3, GTF2H4, GTF2H5); CDK activating kinase (CDK7, CCNH); cyclin G1-interacting protein (MNAT1); DNA

excision repair protein ERCC-51; excision repair cross-complementing 1 (ERCC1); DNA ligase 1 (LIG1); ATP-dependent helicase (ERCC6); and the like.

Also suitable may be DNA repair enzymes in the category that facilitate homologous recombination and include, but are not limited to DNA repair protein RAD51 homolog (RAD51, RAD51L1, RAD51B etc.); DNA repair protein XRCC2; DNA repair protein XRCC3; DNA repair protein RAD52; ATPase (RAD50); 3' exonuclease (MRE11A); and so on.

DNA repair enzymes that are DNA polymerases are also suitable and include DNA polymerase beta subunit (POLB); DNA polymerase gamma (POLG); DNA polymerase subunit delta (POLD1); DNA polymerase II subunit A (POLE); DNA polymerase delta auxiliary protein (PCNA); DNA polymerase zeta (POLZ); MAD2 homolog ((REV7); DNA polymerase eta (POLH); DNA polymerase kappa (POLK); and the like.

Various types of DNA repair enzymes that are often referred to as "editing and processing nucleases" include 3'-nuclease; 3'-exonuclease; 5'-exonuclease; endonuclease; and the like.

Other examples of DNA repair enzymes include DNA helicases including such as ATP DNA helicase and so on.

The DNA repair enzymes may be present as components of botanical extracts, bacterial lysates, biological materials, and the like. For example, botanical extracts may contain DNA repair enzymes.

The compositions of the invention may contain one or more DNA repair enzymes.

#### IV. Other Ingredients

##### A. Proteasome Activator

The composition may contain one more proteasome activators in amounts ranging from about 0.0001 to 65%, preferably from about 0.0005 to 50%, more preferably from about 0.001 to 40%.

Suitable proteasome activators are any compounds, molecules, or active ingredients that stimulate proteasome activity in the cells of keratin surfaces.

5 Examples of suitable proteasome activators include, but are not limited to, algin, alginates, hydrolyzed algin, molasses extract, *Trametes* extracts, including extracts from *Trametes versicolor*, olea hydroxol.

10 The composition of the invention may be in the form of an emulsion, aqueous solution or dispersion, gel, or anhydrous composition. If in the form of an emulsion, it may be a water in oil or oil in water emulsion. If in the form of an emulsion, the composition may contain from about 1-99%, preferably from about 5-90%, more preferably from about 10-85% water and from about 1-99%, preferably from about 5-90%, more preferably from about 5-75% of oil. If in the form of an aqueous suspension or dispersion, the composition may generally contain from about 1-99.9%, preferably from about 5-95%, more preferably from about 10-15 90% water, with the remaining ingredients being the active ingredients or other formula ingredients.

The composition may additionally contain other ingredients including but not limited to those set forth herein.

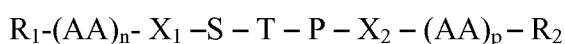
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### B. CLOCK, PER1 Gene Activator

The composition of the invention may contain a CLOCK or PER1 cellular gene activator. Suggested ranges are from about 0.000001 to about 40%, preferably from about 0.000005 to 35%, more preferably from about 0.00001 to 25%. Suitable CLOCK or PER1 25 activators may be present in the form of botanical extracts, polypeptides, peptides, amino acids, and the like.

#### 1. Peptide CLOCK or PER1 Gene Activator

30 A particularly preferred CLOCK and/or PER1 gene activator comprises a peptide of the formula (I):



Where  $(AA)_n-X_1-S-T-P-X_2-(AA)_p$  is (SEQ ID No. 1) and:

$X_1$  represents a threonine, a serine, or is equal to zero,

X<sub>2</sub> represents an isoleucine, leucine, proline, valine, alanine, glycine, or is equal to zero,

AA represents any amino acid or derivative thereof, and n and p are whole numbers between 0 and 4,

5 R<sub>1</sub> represents the primary amine function of the N-terminal amino acid, either free or substituted by a protective grouping that may be chosen from either an acetyl group, a benzoyl group, a tosyl group, or a benzyloxycarbonyl group,

10 R<sub>2</sub> represents the hydroxyl group of the carboxyl function of the C-terminal amino acid, substituted by a protective grouping that may be chosen from either a C<sub>1</sub> to C<sub>20</sub> alkyl chain or an NH<sub>2</sub>, NHY, or NYY group with Y representing a C<sub>1</sub> to C<sub>4</sub> alkyl chain,

wherein the sequence of general formula (I) comprises from about 3 to 13 amino acid residues,

15 said sequence of general formula (I) possibly containing substitutions of amino acids X<sub>1</sub> and X<sub>2</sub> with other chemically equivalent amino acids;

wherein the amino acids are:

Alanine (A)

Arginine (R)

20 Asparagine (N)

Aspartic Acid (D)

Cysteine (C)

Glutamic Acid (E)

Glutamine (Q)

25 Glycine (G)

Histidine (H)

Isoleucine (I)

Leucine (L)

Lysine (K)

30 Methionine (M)

Phenylalanine (F)

Proline (P)

Serine (S)

Threonine (T)

Tryptophan (W)

Tyrosine (Y)

Valine (V)

5 More preferred are peptides of the above formula as follows:

S -T -P -NH<sub>2</sub>

Ser-Thr-Pro-NH<sub>2</sub>

10 (SEQ ID No. 2) Y - V - S -T -P - Y- N- NH<sub>2</sub>

Tyr-Val-Ser-Thr-Pro-Tyr-Asn-NH<sub>2</sub>

(SEQ ID NO. 3) NH<sub>2</sub> -V -S -T -P -E - NH<sub>2</sub>

NH<sub>2</sub>-Val-Ser-Thr-Pro-Glu-NH<sub>2</sub>

15

(SEQ ID No. 4) NH<sub>2</sub> -L -H -S -T- P - P - NH<sub>2</sub>

NH<sub>2</sub>-Leu-His-Ser-Thr-Pro-Pro-NH<sub>2</sub>

(SEQ ID No. 5) CH<sub>3</sub>NH -R -H -S -T -P -E - NH<sub>2</sub>

20

CH<sub>3</sub>-NH-Arg-His-Ser-Thr-Pro-Glu-NH<sub>2</sub>

(SEQ ID No. 6) CH<sub>3</sub>NH - H -S -T -P -E - CH<sub>3</sub>NH

CH<sub>3</sub>-NH-His-Ser-Thr-Pro-Glu-CH<sub>3</sub>-NH

25 More preferred is the S-T-P-NH<sub>2</sub> peptide, SEQ ID No. 4, or mixtures thereof.

Most preferred is a peptide manufactured by ISP-Vinscience under the trademark Chronolux® having the INCI name Tripeptide-32 or Chronogen® having the INCI name Tetrapeptide-26, which has an amino acid sequence of:

30

(SEQ ID No. 7) S-P-L-Q-NH<sub>2</sub>

Ser-Pro-Leu-Gln-NH<sub>2</sub>

## 2. Botanical Extracts

Also suitable as the CLOCK or PER1 gene activator is cichoric acid or isomers or derivatives thereof. Cichoric acid may be synthetic or naturally derived. Synthetic cichoric acid may be purchased from a number of commercial manufacturers including Sigma Aldrich. Cichoric acid may also be extracted from botanical sources that are known to contain cichoric acid such as *Echinacea*, *Cichorium*, *Taraxacum*, *Ocimum*, *Melissa*, or from algae or sea grasses. More specifically, botanical extracts such as *Echinacea purpurea*, *Cichorium intybus*, *Taraxacum officinale*, *Ocimum basilicum*, or *Melissa officinalis*. The term "cichoric acid" when used herein also includes any isomers thereof that are operable to increase PER1 gene expression in skin cells.

A specific example includes a botanical extract from *Echinacea purpurea* sold by Symrise under the brand name Symfinit<sup>TM</sup> 1298 which is an extract of *Echinacea purpurea* which is standardized during the extraction process to contain about 3% by weight of the total extract composition of cichoric acid. *Echinacea* extracts from different sources will vary in cichoric acid content, and as such will yield variable results in induction of PER1 gene expression. For example, we have observed that another component commonly found in extracts of *Echinacea*, specifically caftaric acid, does not increase PER1 gene expression in skin cells. Moreover, each species of *Echinacea* will differ in content of phenolic and cichoric acids. Ethanolic extract of the roots of *Echinacea purpura* will provide more cichoric acid than ethanolic extracts of *Echineacea angustifolia* or *Echinacea pallida*. The content of active ingredients in any extract is also very dependent on the method of extraction. For example, it is known that in many cases enzymatic browning during the extraction process will reduce the phenolic acid content of the resulting extract.

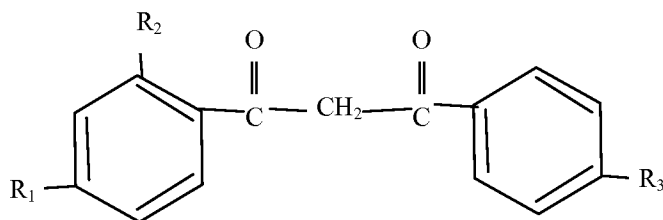
## C. Sunscreens

It may also be desirable to include one or more sunscreens in the compositions of the invention. Such sunscreens include chemical UVA or UVB sunscreens or physical sunscreens in the particulate form. Inclusion of sunscreens in the compositions containing the whitening active ingredient will provide additional protection to skin during daylight hours and promote the effectiveness of the whitening active ingredient on the skin. If present, the sunscreens may

range from about 0.1 to 50%, preferably from about 0.5 to 40%, more preferably from about 1 to 35%.

### 1. UVA Chemical Sunscreens

If desired, the composition may comprise one or more UVA sunscreens. The term "UVA sunscreen" means a chemical compound that blocks UV radiation in the wavelength range of about 320 to 400 nm. Preferred UVA sunscreens are dibenzoylmethane compounds of the formula:

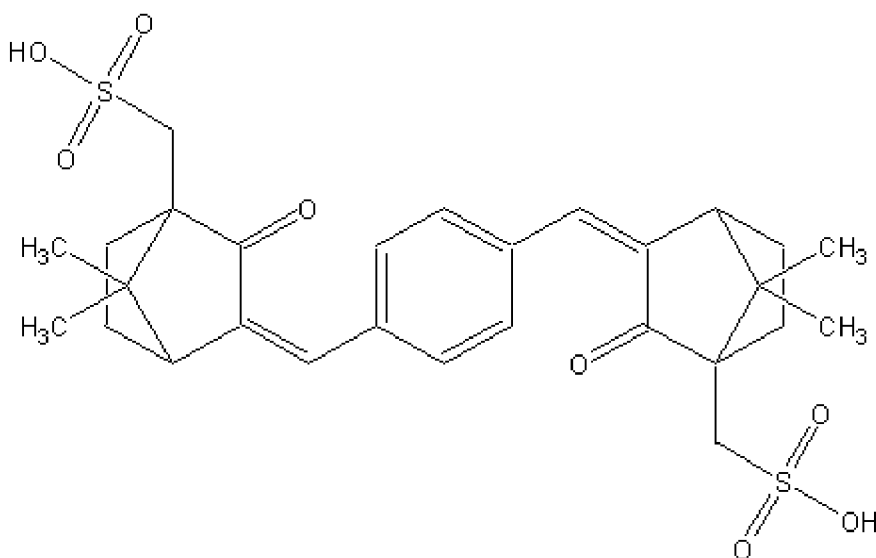


wherein R<sub>1</sub> is H, OR and NRR wherein each R is independently H, C<sub>1-20</sub> straight or branched chain alkyl; R<sub>2</sub> is H or OH; and R<sub>3</sub> is H, C<sub>1-20</sub> straight or branched chain alkyl.

Preferred is where R<sub>1</sub> is OR where R is a C<sub>1-20</sub> straight or branched alkyl, preferably methyl; R<sub>2</sub> is H; and R<sub>3</sub> is a C<sub>1-20</sub> straight or branched chain alkyl, more preferably, butyl.

Examples of suitable UVA sunscreen compounds of this general formula include 4-methyldibenzoylmethane, 2-methyldibenzoylmethane, 4-isopropyldibenzoylmethane, 4-tert-butylidibenzoylmethane, 2,4-dimethyldibenzoylmethane, 2,5-dimethyldibenzoylmethane, 4,4'-diisopropylbenzoylmethane, 4-tert-butyl-4'-methoxydibenzoylmethane, 4,4'-diisopropylbenzoylmethane, 2-methyl-5-isopropyl-4'-methoxydibenzoylmethane, 2-methyl-5-tert-butyl-4'-methoxydibenzoylmethane, and so on. Particularly preferred is 4-tert-butyl-4'-methoxydibenzoylmethane, also referred to as Avobenzone. Avobenzone is commercially available from Givaudan-Roure under the trademark Parsol® 1789, and Merck & Co. under the tradename Eusolex® 9020.

Other types of UVA sunscreens include dicamphor sulfonic acid derivatives, such as ecamsule, a sunscreen sold under the trade name Mexoryl®, which is terephthalylidene dicamphor sulfonic acid, having the formula:

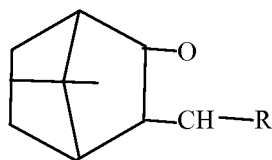


The composition may contain from about 0.001-20%, preferably 0.005-5%, more preferably about 0.005-3% by weight of the composition of UVA sunscreen. In the preferred embodiment of the invention the UVA sunscreen is Avobenzone, and it is present at not greater than about 3% by weight of the total composition.

## 2. UVB Chemical Sunscreens

The term "UVB sunscreen" means a compound that blocks UV radiation in the wavelength range of from about 290 to 320 nm. A variety of UVB chemical sunscreens exist including alpha-cyano-beta,beta-diphenyl acrylic acid esters as set forth in U.S. Pat. No. 3,215,724, which is hereby incorporated by reference in its entirety. One particular example of an alpha-cyano-beta,beta-diphenyl acrylic acid ester is Octocrylene, which is 2-ethylhexyl 2-cyano-3,3-diphenylacrylate. In certain cases the composition may contain no more than about 10% by weight of the total composition of octocrylene. Suitable amounts range from about 0.001-10% by weight. Octocrylene may be purchased from BASF under the tradename Uvinul® N-539.

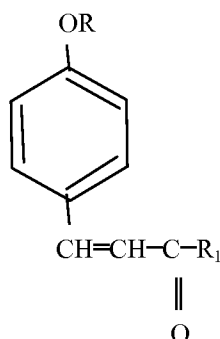
Other suitable sunscreens include benzylidene camphor derivatives as set forth in U.S. Pat. No. 3,781,417, which is hereby incorporated by reference in its entirety. Such benzylidene camphor derivatives have the general formula:



5 wherein R is p-tolyl or styryl, preferably styryl. Particularly preferred is 4-methylbenzylidene camphor, which is a lipid soluble UVB sunscreen compound sold under the tradename Eusolex 6300 by Merck.

Also suitable are cinnamate derivatives having the general formula:

10



15

wherein R and R<sub>1</sub> are each independently a C<sub>1-20</sub> straight or branched chain alkyl. Preferred is where R is methyl and R<sub>1</sub> is a branched chain C<sub>1-10</sub>, preferably C<sub>8</sub> alkyl. The preferred compound is ethylhexyl methoxycinnamate, also referred to as Octoxinate or octyl methoxycinnamate. The compound may be purchased from Givaudan Corporation under the tradename Parsol® MCX, or BASF under the tradename Uvinul® MC 80.

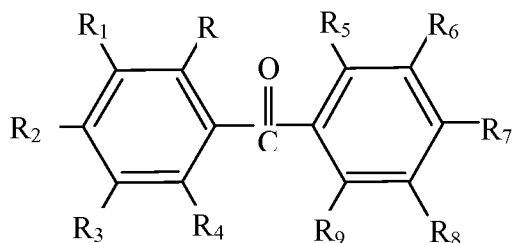
20

Also suitable are mono-, di-, and triethanolamine derivatives of such methoxy cinnamates including diethanolamine methoxycinnamate. Cinoxate, the aromatic ether derivative of the above compound is also acceptable. If present, the Cinoxate should be found at no more than about 3% by weight of the total composition.

25

Also suitable as UVB screening agents are various benzophenone derivatives having the general formula:

30

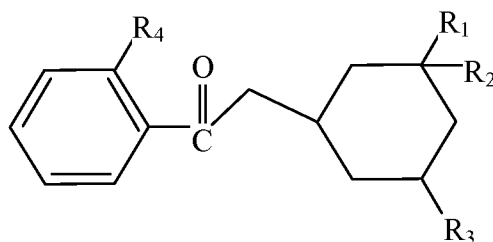


5

wherein R through R<sub>9</sub> are each independently H, OH, NaO<sub>3</sub>S, SO<sub>3</sub>H, SO<sub>3</sub>Na, Cl, R", OR" where R" is C<sub>1-20</sub> straight or branched chain alkyl. Examples of such compounds include Benzophenone 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12. Particularly preferred is where the benzophenone derivative is Benzophenone 3 (also referred to as Oxybenzone), Benzophenone 4 (also referred to as Sulisobenzone), Benzophenone 5 (Sulisobenzone Sodium), and the like. Most preferred is Benzophenone 3.

Also suitable are certain menthyl salicylate derivatives having the general formula:

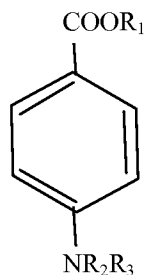
15



wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are each independently H, OH, NH<sub>2</sub>, or C<sub>1-20</sub> straight or branched chain alkyl. Particularly preferred is where R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub> are methyl and R<sub>4</sub> is hydroxyl or NH<sub>2</sub>, the compound having the name homomenthyl salicylate (also known as Homosalate) or menthyl anthranilate. Homosalate is available commercially from Merck under the trademark Eusolex® HMS and menthyl anthranilate is commercially available from Haarmann & Reimer under the trademark Heliopan®. If present, the Homosalate should be found at no more than about 15% by weight of the total composition.

30

Various amino benzoic acid derivatives are suitable UVB absorbers including those having the general formula:



5

wherein R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub> are each independently H, C<sub>1-20</sub> straight or branched chain alkyl which may be substituted with one or more hydroxy groups. Particularly preferred is wherein R<sub>1</sub> is H or C<sub>1-8</sub> straight or branched alkyl, and R<sub>2</sub> and R<sub>3</sub> are H, or C<sub>1-8</sub> straight or branched chain alkyl. Particularly preferred are PABA, ethyl hexyl dimethyl PABA (Padimate O), ethyldihydroxypropyl PABA, and the like. If present Padimate O should be found at no more than about 8% by weight of the total composition.

10

15

Salicylate derivatives are also acceptable UVB absorbers. Such compounds have the general formula: wherein R is a straight or branched chain alkyl, including derivatives of the above compound formed from mono-, di-, or triethanolamines. Particular preferred are octyl salicylate, TEA-salicylate, DEA-salicylate, and mixtures thereof.

Generally, the amount of the UVB chemical sunscreen present may range from about 0.001-45%, preferably 0.005-40%, more preferably about 0.01-35% by weight of the total composition.

20

If desired, the compositions of the invention may be formulated to have certain SPF (sun protective factor) values ranging from about 1-50, preferably about 2-45, most preferably about 5-30. Calculation of SPF values is well known in the art.

25

#### D. Surfactants

It may be desirable for the composition to contain one more surfactants, especially if in the emulsion form. However, such surfactants may be used if the compositions are solutions, suspensions, or anhydrous also, and will assist in dispersing ingredients that have polarity, for example pigments. Such surfactants may be silicone or organic based. The surfactants will also aid in the formation of stable emulsions of either the water-in-oil or oil-in-water form. If present, the surfactant may range from about 0.001 to 30%, preferably from about 0.005 to 25%, more preferably from about 0.1 to 20% by weight of the total composition.

30

### 1. Organic Nonionic Surfactants

The composition may comprise one or more nonionic organic surfactants. Suitable nonionic surfactants include alkoxyated alcohols or ethers, formed by the reaction of an alcohol with an alkylene oxide, usually ethylene or propylene oxide. Suitable alcohols include mono-, di-, or polyhydric short chain (C1-6) alcohols; aromatic or aliphatic saturated or unsaturated fatty (C12-40) alcohols, of cholesterol; and so on.

In one embodiment the alcohol is cholesterol, or an aromatic or aliphatic saturated or unsaturated fatty alcohol which may have from 6 to 40, preferably from about 10 to 30, more preferably from about 12 to 22 carbon atoms. Examples include oleyl alcohol, cetearyl alcohol, cetyl alcohol, stearyl alcohol, isostearyl alcohol, behenyl alcohol, and the like. Examples of such ingredients include Oleth 2-100; Steareth 2-100; Beheneth 5-30; Cetareth 2-100; Ceteth 2-100; Choleth 2-100 wherein the number range means the number of repeating ethylene oxide units, e.g. Ceteth 2-100 means Ceteth where the number of repeating ethylene oxide units ranges from 2 to 100. Derivatives of alkoxyated alcohols are also suitable, such as phosphoric acid esters thereof.

Some preferred organic nonionic surfactants include Oleth-3, Oleth-5, Oleth-3 phosphate, Choleth-24; Ceteth-24; and so on.

Also suitable are alkoxyated alcohols formed with mono-, di-, or polyhydric short chain alcohols, for example those having from about 1 to 6 carbon atoms. Examples include glucose, glycerin, or alkylated derivatives thereof. Examples include glycereth 2-100; gluceth 2-100; methyl gluceth 2-100 and so on. More preferred are methyl gluceth-20; glycereth-26 and the like.

Other types of alkoxyated alcohols are suitable surfactants, including ethylene oxide polymers having varying numbers of repeating EO groups, generally referred to as PEG 12 to 200. More preferred are PEG-75, which is may be purchased from Dow Chemical under the trade name Carbowax PEG-3350.

Other suitable nonionic surfactants include alkoxyated sorbitan and alkoxyated sorbitan derivatives. For example, alkoxylation, in particular ethoxylation of sorbitan provides polyalkoxyated sorbitan derivatives. Esterification of polyalkoxyated sorbitan provides

sorbitan esters such as the polysorbates. For example, the polyalkoxyolated sorbitan can be esterified with C6-30, preferably C12-22 fatty acids. Examples of such ingredients include Polysorbates 20-85, sorbitan oleate, sorbitan sesquioleate, sorbitan palmitate, sorbitan sesquiosostearate, sorbitan stearate, and so on.

5

## 2. Silicone or Silane Surfactants

Also suitable are various types of silicone or silane-based surfactants. Examples include organosiloxanes substituted with ethylene oxide or propylene oxide groups such as PEG dimethicones which are dimethicones substituted with polyethylene glycols including those having the INCI names PEG-1 dimethicone; PEG-4 dimethicone; PEG-8 dimethicone; PEG-12 dimethicone; PEG-20 dimethicone; and so on.

10

15

Also suitable are silanes substituted with ethoxy groups or propoxy groups or both, such as various types of PEG methyl ether silanes such as bis-PEG-18 methyl ether dimethyl silane; and so on.

Further examples of silicone based surfactants include those having the generic names dimethicone copolyol; cetyl dimethicone copolyol; and so on.

20

## F. Botanical Extracts

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It may be desirable to incorporate one more additional botanical extracts into the composition. If present suggested ranges are from about 0.0001 to 20%, preferably from about 0.0005 to 15%, more preferably from about 0.001 to 10%. Suitable botanical extracts include extracts from plants (herbs, roots, flowers, fruits, seeds) such as flowers, fruits, vegetables, and so on, including yeast ferment extract, *Padina Pavonica* extract, *Thermus Thermophilis* ferment extract, *Camelina Sativa* seed oil, *Boswellia Serrata* extract, olive extract, *Acacia Dealbata* extract, *Acer Saccharinum* (sugar maple), *Acidopholus*, *Acorus*, *Aesculus*, *Agaricus*, *Agave*, *Agrimonia*, algae, aloe, citrus, *Brassica*, cinnamon, orange, apple, blueberry, cranberry, peach, pear, lemon, lime, pea, seaweed, caffeine, green tea, chamomile, willowbark, mulberry, poppy, and those set forth on pages 1646 through 1660 of the CTFA Cosmetic Ingredient Handbook, Eighth Edition, Volume 2. Further specific examples include, but are not limited to, *Glycyrrhiza Glabra*, *Salix Nigra*, *Macrocystis Pyrifer*, *Pyrus Malus*, *Saxifraga Sarmentosa*, *Vitis Vinifera*, *Morus Nigra*, *Scutellaria Baicalensis*, *Anthemis Nobilis*, *Salvia Sclarea*, *Rosmarinus Officianalis*, *Citrus Medica Limonum*, *Panax Ginseng*,

*Siegesbeckia Orientalis*, *Fructus Mume*, *Ascophyllum Nodosum*, *Glycine Soja* extract, *Beta Vulgaris*, *Haberlea Rhodopensis*, *Polygonum Cuspidatum*, *Citrus Aurantium Dulcis*, *Vitis Vinifera*, *Selaginella Tamariscina*, *Humulus Lupulus*, *Citrus Reticulata Peel*, *Punica Granatum*, *Asparagopsis*, *Curcuma Longa*, *Menyanthes Trifoliata*, *Helianthus Annuus*,

5 *Hordeum Vulgare*, *Cucumis Sativus*, *Evernia Prunastri*, *Evernia Furfuracea*, *Kola Acuminata*, and mixtures thereof. If desired such botanical extracts may be fermented to increase potency or activity. Fermentation may be accomplished by standard fermentation techniques using bacteria or yeast.

#### 10 G. Biological Materials

Also suitable are various types of biological materials such as those derived from cells, fermented materials, and so on. If present such materials may range from about 0.001 to 30%, preferably from about 0.005 to 25%, more preferably from about 0.01 to 20%. Examples include fragments of cellular RNA or DNA, probiotic microorganisms, or ferments of  
15 microorganisms and organic materials from plants such as leaves, seeds, extracts, flowers, etc. Particularly preferred are RNA fragments.

#### H. Aqueous Phase Structuring Agent

In the case where the compositions are in the form of aqueous solutions, dispersions or  
20 emulsions, in addition to water the aqueous phase may contain one or more aqueous phase structuring agents, that is, an agent that increases the viscosity or, or thickens, the aqueous phase of the composition. This is particularly desirable when the composition is in the form of a serum or gel. Suitable ranges of aqueous phase structuring agent, if present, are from about 0.01 to 30%, preferably from about 0.1 to 20%, more preferably from about 0.5 to 15% by  
25 weight of the total composition. Examples of such agents include various acrylate based thickening agents, natural or synthetic gums, polysaccharides, and the like, including but not limited to those set forth below.

##### 1. Polysaccharides

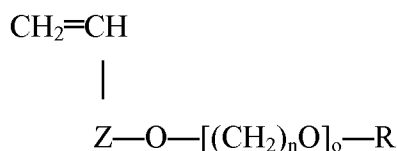
30 Polysaccharides may be suitable aqueous phase thickening agents. Examples of such polysaccharides include naturally derived materials such as agar, agarose, alicaligenes polysaccharides, algin, alginic acid, acacia gum, amylopectin, chitin, dextran, cassia gum,

cellulose gum, gelatin, gellan gum, hyaluronic acid, hydroxyethyl cellulose, methyl cellulose, ethyl cellulose, pectin, sclerotium gum, xanthan gum, pectin, trehalose, gelatin, and so on.

## 2. Acrylate Polymers

5 Also suitable are different types of synthetic polymeric thickeners. One type includes acrylic polymeric thickeners comprised of monomers A and B wherein A is selected from the group consisting of acrylic acid, methacrylic acid, and mixtures thereof; and B is selected from the group consisting of a C<sub>1-22</sub> alkyl acrylate, a C<sub>1-22</sub> alky methacrylate, and mixtures thereof are suitable. In one embodiment the A monomer comprises one or more of acrylic acid or  
10 methacrylic acid, and the B monomer is selected from the group consisting of a C<sub>1-10</sub>, most preferably C<sub>1-4</sub> alkyl acrylate, a C<sub>1-10</sub>, most preferably C<sub>1-4</sub> alkyl methacrylate, and mixtures thereof. Most preferably the B monomer is one or more of methyl or ethyl acrylate or methacrylate. The acrylic copolymer may be supplied in an aqueous solution having a solids content ranging from about 10-60%, preferably 20-50%, more preferably 25-45% by weight of  
15 the polymer, with the remainder water. The composition of the acrylic copolymer may contain from about 0.1-99 parts of the A monomer, and about 0.1-99 parts of the B monomer. Acrylic polymer solutions include those sold by Seppic, Inc., under the tradename Capigel.

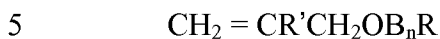
Also suitable are acrylic polymeric thickeners that are copolymers of A, B, and C  
20 monomers wherein A and B are as defined above, and C has the general formula:



25 wherein Z is -(CH<sub>2</sub>)<sub>m</sub>; wherein m is 1-10, n is 2-3, o is 2-200, and R is a C<sub>10-30</sub> straight or branched chain alkyl. Examples of the secondary thickening agent above, are copolymers where A and B are defined as above, and C is CO, and wherein n, o, and R are as above defined. Examples of such secondary thickening agents include acrylates/steareth-20  
30 methacrylate copolymer, which is sold by Rohm & Haas under the tradename Acrysol ICS-1.

Also suitable are acrylate based anionic amphiphilic polymers containing at least one hydrophilic unit and at least one allyl ether unit containing a fatty chain. Preferred are those

where the hydrophilic unit contains an ethylenically unsaturated anionic monomer, more specifically a vinyl carboxylic acid such as acrylic acid, methacrylic acid or mixtures thereof, and where the allyl ether unit containing a fatty chain corresponds to the monomer of formula:



in which R' denotes H or CH<sub>3</sub>, B denotes the ethylenoxy radical, n is zero or an integer ranging from 1 to 100, R denotes a hydrocarbon radical selected from alkyl, arylalkyl, aryl, alkylaryl and cycloalkyl radicals which contain from 8 to 30 carbon atoms, preferably from 10 to 24, and even more particularly from 12 to 18 carbon atoms. More preferred in this case is where R' denotes H, n is equal to 10 and R denotes a stearyl (C18) radical. Anionic amphiphilic polymers of this type are described and prepared in U.S. Patent Nos. 4,677,152 and 4,702,844, both of which are hereby incorporated by reference in their entirety. Among these anionic amphiphilic polymers, polymers formed of 20 to 60% by weight acrylic acid and/or  
10 methacrylic acid, of 5 to 60% by weight lower alkyl methacrylates, of 2 to 50% by weight allyl ether containing a fatty chain as mentioned above, and of 0 to 1% by weight of a crosslinking agent which is a well-known copolymerizable polyethylenic unsaturated monomer, for instance diallyl phthalate, allyl (meth)acrylate, divinylbenzene, (poly)ethylene glycol dimethacrylate and methylenebisacrylamide. One commercial example of such  
20 polymers are crosslinked terpolymers of methacrylic acid, of ethyl acrylate, of polyethylene glycol (having 10 EO units) ether of stearyl alcohol or steareth-10, in particular those sold by the company Allied Colloids under the names *SALCARE* SC80 and *SALCARE* SC90, which are aqueous emulsions containing 30% of a crosslinked terpolymer of methacrylic acid, of ethyl acrylate and of steareth-10 allyl ether (40/50/10).

25            Also suitable are acrylate copolymers such as Polyacrylate-3 which is a copolymer of methacrylic acid, methylmethacrylate, methylstyrene isopropylisocyanate, and PEG-40 behenate monomers; Polyacrylate-10 which is a copolymer of sodium acryloyldimethyltaurate, sodium acrylate, acrylamide and vinyl pyrrolidone monomers; or  
30 Polyacrylate-11, which is a copolymer of sodium acryloyldimethylacryloyldimethyl taurate, sodium acrylate, hydroxyethyl acrylate, lauryl acrylate, butyl acrylate, and acrylamide monomers.

Also suitable are crosslinked acrylate based polymers where one or more of the acrylic groups may have substituted long chain alkyl (such as 6-40, 10-30, and the like) groups, for example acrylates/C<sub>10-30</sub> alkyl acrylate crosspolymer which is a copolymer of C<sub>10-30</sub> alkyl acrylate and one or more monomers of acrylic acid, methacrylic acid, or one of their simple esters crosslinked with the allyl ether of sucrose or the allyl ether of pentaerythritol. Such polymers are commonly sold under the Carbopol or Pemulen tradenames and have the CTFA name carbomer.

Also suitable are acrylate based polymeric thickeners sold by Clariant under the Aristoflex trademark such as Aristoflex AVC, which is ammonium acryloyldimethyltaurate/VP copolymer; Aristoflex AVL which is the same polymer has found in AVC dispersed in mixture containing caprylic/capric triglyceride, trilaureth-4, and polyglyceryl-2 sesquiisostearate; or Aristoflex HMB which is ammonium acryloyldimethyltaurate/behent-25 methacrylate crosspolymer, and the like.

### 3. Alkylene Glycols

Also suitable as the aqueous phase thickening agents are various polyethylene glycols (PEG) derivatives where the degree of polymerization ranges from 1,000 to 200,000. Such ingredients are indicated by the designation "PEG" followed by the degree of polymerization in thousands, such as PEG-45M, which means PEG having 45,000 repeating ethylene oxide units. Examples of suitable PEG derivatives include PEG 2M, 5M, 7M, 9M, 14M, 20M, 23M, 25M, 45M, 65M, 90M, 115M, 160M, 180M, and the like.

Also suitable are polyglycerins which are repeating glycerin moieties where the number of repeating moieties ranges from 15 to 200, preferably from about 20-100. Examples of suitable polyglycerins include those having the CFTA names polyglycerin-20, polyglycerin-40, and the like.

#### I. Humectants

The composition may contain one or more humectants. If present, they may range from about 0.01 to 75%, preferably from about 0.5 to 70%, more preferably from about 0.5 to 40%. Examples of suitable humectants include glycols, sugars, and the like. Suitable glycols are in monomeric or polymeric form and include polyethylene and polypropylene glycols such

as PEG 4-10, which are polyethylene glycols having from 4 to 10 repeating ethylene oxide units; as well as C<sub>1-6</sub> alkylene glycols such as propylene glycol, butylene glycol, pentylene glycol, and the like. Suitable sugars, some of which are also polyhydric alcohols, are also suitable humectants. Examples of such sugars include glucose, fructose, honey, hydrogenated  
 5 honey, inositol, maltose, mannitol, maltitol, sorbitol, sucrose, xylitol, xylose, and so on. Also suitable is urea. Preferably, the humectants used in the composition of the invention are C<sub>1-6</sub>, preferably C<sub>2-4</sub> alkylene glycols, most particularly butylene glycol.

### J. Oils

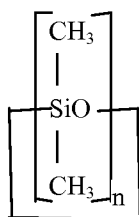
10 In the event the compositions of the invention are in emulsion form, the composition will comprise an oil phase. Oily ingredients are desirable for the skin moisturizing and protective properties. Suitable oils include silicones, esters, vegetable oils, synthetic oils, including but not limited to those set forth herein. The oils may be volatile or nonvolatile, and are preferably in the form of a pourable liquid at room temperature. The term "volatile" means  
 15 that the oil has a measurable vapor pressure, or a vapor pressure of at least about 2 mm. of mercury at 20° C. The term "nonvolatile" means that the oil has a vapor pressure of less than about 2 mm. of mercury at 20° C. If present, such oils may range from about 0.01 to 85%, preferably from about 0.05 to 80%, more preferably from about 0.1 to 50%.

#### 20 1. Volatile Oils

Suitable volatile oils generally have a viscosity ranging from about 0.5 to 5 centistokes 25° C. and include linear silicones, cyclic silicones, paraffinic hydrocarbons, or mixtures thereof.

##### (a). Volatile Silicones

25 Cyclic silicones are one type of volatile silicone that may be used in the composition. Such silicones have the general formula:



30

where n=3-6, preferably 4, 5, or 6.

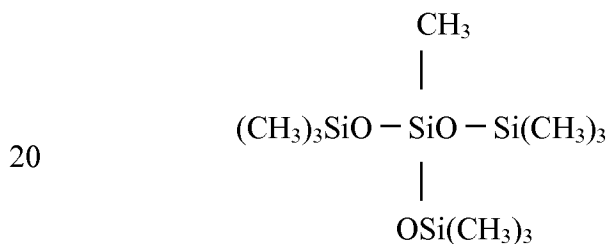
Also suitable are linear volatile silicones, for example, those having the general formula:



5 where  $n=0, 1, 2, 3, 4,$  or  $5,$  preferably  $0, 1, 2, 3,$  or  $4.$

Cyclic and linear volatile silicones are available from various commercial sources including Dow Corning Corporation and General Electric. The Dow Corning linear volatile  
10 silicones are sold under the tradenames Dow Corning 244, 245, 344, and 200 fluids. These fluids include hexamethyldisiloxane (viscosity 0.65 centistokes (abbreviated cst)), octamethyltrisiloxane (1.0 cst), decamethyltetrasiloxane (1.5 cst), dodecamethyl-pentasiloxane (2 cst) and mixtures thereof, with all viscosity measurements being at 25° C.

15 Suitable branched volatile silicones include alkyl trimethicones such as methyl trimethicone having the general formula:



Methyl trimethicone may be purchased from Shin-Etsu Silicones under the tradename TMF-  
25 1.5, having a viscosity of 1.5 centistokes at 25° C.

#### (b). Volatile Paraffinic Hydrocarbons

Also suitable as the volatile oils are various straight or branched chain paraffinic hydrocarbons having 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms, more preferably 8 to 16 carbon atoms. Suitable hydrocarbons include pentane, hexane,  
30 heptane, decane, dodecane, tetradecane, tridecane, and C<sub>8-20</sub> isoparaffins as disclosed in U.S. Pat. Nos. 3,439,088 and 3,818,105, both of which are hereby incorporated by reference. Preferred volatile paraffinic hydrocarbons have a molecular weight of 70-225, preferably 160 to 190 and a boiling point range of 30 to 320, preferably 60 to 260° C., and a viscosity of less

than about 10 cst. at 25° C. Such paraffinic hydrocarbons are available from EXXON under the ISOPARS trademark, and from the Permethyl Corporation. Suitable C<sub>12</sub> isoparaffins are manufactured by Permethyl Corporation under the tradename Permethyl 99A. Various C<sub>16</sub> isoparaffins commercially available, such as isohexadecane (having the tradename Permethyl R), are also suitable.

## 2. Non-Volatile Oils

A variety of nonvolatile oils are also suitable for use in the compositions of the invention. The nonvolatile oils generally have a viscosity of greater than about 5 to 10 centistokes at 25° C., and may range in viscosity up to about 1,000,000 centipoise at 25° C. Examples of nonvolatile oils include, but are not limited to:

### (a). Esters

Suitable esters are mono-, di-, and triesters. The composition may comprise one or more esters selected from the group, or mixtures thereof.

#### (i). Monoesters

Monoesters are defined as esters formed by the reaction of a monocarboxylic acid having the formula R-COOH, wherein R is a straight or branched chain saturated or unsaturated alkyl having 2 to 45 carbon atoms, or phenyl; and an alcohol having the formula R-OH wherein R is a straight or branched chain saturated or unsaturated alkyl having 2-30 carbon atoms, or phenyl. Both the alcohol and the acid may be substituted with one or more hydroxyl groups. Either one or both of the acid or alcohol may be a "fatty" acid or alcohol, and may have from about 6 to 30 carbon atoms, more preferably 12, 14, 16, 18, or 22 carbon atoms in straight or branched chain, saturated or unsaturated form. Examples of monoester oils that may be used in the compositions of the invention include hexyl laurate, butyl isostearate, hexadecyl isostearate, cetyl palmitate, isostearyl neopentanoate, stearyl heptanoate, isostearyl isononanoate, stearyl lactate, stearyl octanoate, stearyl stearate, isononyl isononanoate, and so on.

#### (ii). Diesters

Suitable diesters are the reaction product of a dicarboxylic acid and an aliphatic or aromatic alcohol or an aliphatic or aromatic alcohol having at least two substituted hydroxyl

groups and a monocarboxylic acid. The dicarboxylic acid may contain from 2 to 30 carbon atoms, and may be in the straight or branched chain, saturated or unsaturated form. The dicarboxylic acid may be substituted with one or more hydroxyl groups. The aliphatic or aromatic alcohol may also contain 2 to 30 carbon atoms, and may be in the straight or  
5 branched chain, saturated, or unsaturated form. Preferably, one or more of the acid or alcohol is a fatty acid or alcohol, i.e. contains 12-22 carbon atoms. The dicarboxylic acid may also be an alpha hydroxy acid. The ester may be in the dimer or trimer form. Examples of diester oils that may be used in the compositions of the invention include diisotearyl malate, neopentyl glycol dioctanoate, dibutyl sebacate, dicetearyl dimer dilinoleate, dicetyl adipate,  
10 diisocetyl adipate, diisononyl adipate, diisostearyl dimer dilinoleate, diisostearyl fumarate, diisostearyl malate, dioctyl malate, and so on.

### (iii). Triesters

Suitable triesters comprise the reaction product of a tricarboxylic acid and an aliphatic  
15 or aromatic alcohol or alternatively the reaction product of an aliphatic or aromatic alcohol having three or more substituted hydroxyl groups with a monocarboxylic acid. As with the mono- and diesters mentioned above, the acid and alcohol contain 2 to 30 carbon atoms, and may be saturated or unsaturated, straight or branched chain, and may be substituted with one or more hydroxyl groups. Preferably, one or more of the acid or alcohol is a fatty acid or  
20 alcohol containing 12 to 22 carbon atoms. Examples of triesters include esters of arachidonic, citric, or behenic acids, such as triarachidin, tributyl citrate, triisostearyl citrate, tri C<sub>12-13</sub> alkyl citrate, tricaprylin, tricaprylyl citrate, tridecyl behenate, trioctyldodecyl citrate, tridecyl behenate; or tridecyl cocoate, tridecyl isononanoate, and so on.

25 Esters suitable for use in the composition are further described in the C.T.F.A. Cosmetic Ingredient Dictionary and Handbook, Eleventh Edition, 2006, under the classification of "Esters", the text of which is hereby incorporated by reference in its entirety.

### (b). Hydrocarbon Oils

30 It may be desirable to incorporate one or more nonvolatile hydrocarbon oils into the composition. Suitable nonvolatile hydrocarbon oils include paraffinic hydrocarbons and olefins, preferably those having greater than about 20 carbon atoms. Examples of such hydrocarbon oils include C<sub>24-28</sub> olefins, C<sub>30-45</sub> olefins, C<sub>20-40</sub> isoparaffins, hydrogenated

polyisobutene, polyisobutene, polydecene, hydrogenated polydecene, mineral oil, pentahydrosqualene, squalene, squalane, and mixtures thereof. In one preferred embodiment such hydrocarbons have a molecular weight ranging from about 300 to 1000 Daltons.

5 (c). Glyceryl Esters of Fatty Acids

Synthetic or naturally occurring glyceryl esters of fatty acids, or triglycerides, are also suitable for use in the compositions. Both vegetable and animal sources may be used.

10 Examples of such oils include castor oil, lanolin oil, C<sub>10-18</sub> triglycerides, caprylic/capric/triglycerides, sweet almond oil, apricot kernel oil, sesame oil, camelina sativa oil, tamanu seed oil, coconut oil, corn oil, cottonseed oil, linseed oil, ink oil, olive oil, palm oil, illipe butter, rapeseed oil, soybean oil, grapeseed oil, sunflower seed oil, walnut oil, and the like.

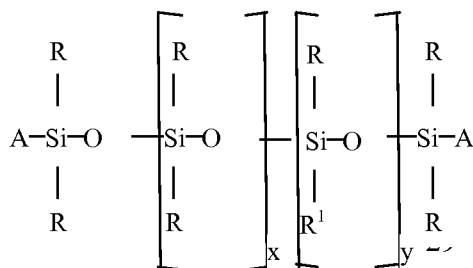
15 Also suitable are synthetic or semi-synthetic glyceryl esters, such as fatty acid mono-, di-, and triglycerides which are natural fats or oils that have been modified, for example, mono-, di- or triesters of polyols such as glycerin. In an example, a fatty (C<sub>12-22</sub>) carboxylic acid is reacted with one or more repeating glyceryl groups. glyceryl stearate, diglyceryl diiosostearate, polyglyceryl-3 isostearate, polyglyceryl-4 isostearate, polyglyceryl-6 ricinoleate, glyceryl dioleate, glyceryl diisostearate, glyceryl tetraisostearate, glyceryl  
20 trioctanoate, diglyceryl distearate, glyceryl linoleate, glyceryl myristate, glyceryl isostearate, PEG castor oils, PEG glyceryl oleates, PEG glyceryl stearates, PEG glyceryl tallowates, and so on.

(d). Nonvolatile Silicones

25 Nonvolatile silicone oils, both water soluble and water insoluble, are also suitable for use in the composition. Such silicones preferably have a viscosity ranging from about greater than 5 to 800,000 cst, preferably 20 to 200,000 cst at 25° C. Suitable water insoluble silicones include amine functional silicones such as amodimethicone.

For example, such nonvolatile silicones may have the following general formula:

30



wherein R and R' are each independently C<sub>1-30</sub> straight or branched chain, saturated or unsaturated alkyl, phenyl or aryl, trialkylsiloxy, and x and y are each independently 1-1,000,000; with the proviso that there is at least one of either x or y, and A is alkyl siloxy endcap unit. Preferred is where A is a methyl siloxy endcap unit; in particular

5 trimethylsiloxy, and R and R' are each independently a C<sub>1-30</sub> straight or branched chain alkyl, phenyl, or trimethylsiloxy, more preferably a C<sub>1-22</sub> alkyl, phenyl, or trimethylsiloxy, most preferably methyl, phenyl, or trimethylsiloxy, and resulting silicone is dimethicone, phenyl dimethicone, diphenyl dimethicone, phenyl trimethicone, or trimethylsiloxyphenyl dimethicone. Other examples include alkyl dimethicones such as cetyl dimethicone, and the

10 like wherein at least one R is a fatty alkyl (C<sub>12</sub>, C<sub>14</sub>, C<sub>16</sub>, C<sub>18</sub>, C<sub>20</sub>, or C<sub>22</sub>), and the other R is methyl, and A is a trimethylsiloxy endcap unit, provided such alkyl dimethicone is a pourable liquid at room temperature. Phenyl trimethicone can be purchased from Dow Corning Corporation under the tradename 556 Fluid. Trimethylsiloxyphenyl dimethicone can be purchased from Wacker-Chemie under the tradename PDM-1000. Cetyl dimethicone, also

15 referred to as a liquid silicone wax, may be purchased from Dow Corning as Fluid 2502, or from DeGussa Care & Surface Specialties under the trade names Abil Wax 9801, or 9814.

#### K. Vitamins and Antioxidants

It may be desirable to incorporate one or more vitamins or antioxidants in the

20 compositions. If present, suggested ranges are from about 0.001 to 20%, preferably from about 0.005 to 15%, more preferably from about 0.010 to 10%. Preferably such vitamins, vitamin derivatives and/or antioxidants are operable to scavenge free radicals in the form of singlet oxygen. Such vitamins may include tocopherol or its derivatives such as tocopherol acetate, tocopherol ferulate; ascorbic acid or its derivatives such as ascorbyl palmitate,

25 magnesium ascorbyl phosphate; Vitamin A or its derivatives such as retinyl palmitate; or vitamins D, K, B, or derivatives thereof.

#### L. Preferred Compositions

Preferred compositions are in the aqueous solution or emulsion form and contain at

30 least one autophagy activator and at least one DNA repair enzyme. The composition may optionally contain at least one CLOCK or PER1 gene activator and/or at least one proteasome activator.

More preferred is where within the composition the autophagy activator is a yeast extract that may be fermented and the DNA repair enzyme is one or more of *Arabidopsis thaliana* or ferment thereof. If present the proteasome activator is algin, hydrolyzed algin, or alginate. If present, the nonionic organic surfactant is an alkoxyated alcohol, the chemical  
5 sunscreen is a UVB sunscreen, the CLOCK or PER1 keratinocyte gene activator is Tripeptide-32 or Tetrapeptide-26, the DNA repair enzyme, if present, is a mixture of *Arabidopsis Thaliana* extract, *Micrococcus* lysate, *Bifida* Ferment lysate, *Lactobacillus* ferment, and Plankton extract, and the at least one oil is an organic ester or hydrocarbon.

## 10 The Method

The invention is also directed to a method for improving normal cellular detoxification processes by treating the cells with a composition that stimulates selective catabolysis by activating or enhancing cellular autophagy processes and/or repairing cellular DNA. In the case where a CLOCK or PER1 gene activator may be present, it may improve the  
15 synchronicity of the metabolic pathways of the treated cells, which in turn will improve the efficiency of the autophagy and/or proteasome activation processes.

In the method of the invention, the composition may be applied to keratin surfaces such as skin, scalp, nails or hair one or more times per day. For example, the composition may be applied to skin in the morning prior to beginning daily activities and/or at night prior  
20 to retiring. The composition may be applied as part of a regimen; that is, the skin is cleansed and treated with toner, after which the composition of the invention is applied. The composition may be part of a kit containing a cleanser, toner, and the composition of the invention.

25 Preferably the composition is applied to the face and/or neck and décolletage prior to retiring to repair or eliminate damaged cellular material and provide general improvement of the skin. Combining the composition of the invention at night prior to retiring maximizes the cellular detoxification processes. In general, treatment of skin with the composition of the invention promotes cellular viability, longevity, and health.

30 The invention will be further described in connection with the following examples which are set forth for the purposes of illustration only.

## EXAMPLE 1

Normal human dermal fibroblasts were harvested and assayed for cellular viability when untreated, treated with solutions containing 0.0025% of DNA repair enzyme *Arabidopsis thaliana* extract, 1% autophagy activator in the form of yeast extract, and a solution containing a mixture of 0.025% *Arabidopsis thaliana* extract and 1% yeast extract autophagy activator.

The fibroblasts were placed in concentrations of 150,000 cells per plate for the 48 hour test, and 300,000 cells per plate for the 24 hour test on 96 well plates. The cells were incubated at 37° C., 5% CO<sub>2</sub>, and 95% humidity for 24 hours. The test compositions were prepared as follows:

(a) Autophagy Activator. 1% Yeast Extract: 300ul of yeast extract was added to 14.7 ml of Media Dulbecco's Modified Eagle Media (DMEM) to create a 2% stock solution. Then 2.5 ml of this stock solution was added to 2.5 ml of DMEM to create 1% by weight solution.

(b) DNA Repair Enzyme. Solution of 0.025% *Arabidopsis thaliana* extract: 15ul of Roxisomes® was combined with 14.985 ml of DMEM and filtered to create a 0.1% stock solution. Then 2.5ml of this stock solution was diluted with 2.5 ml of DMEM to create a 0.05% solution. Then 2.5 ml of this 0.05% solution was diluted with 2.5 ml of DMEM to make a 0.025% solution of the extract.

(c) Autophagy Activator + DNA Repair Enzyme. 5 ml of the 2% yeast extract solution was mixed with 5 ml of the 0.05% solution of Roxisomes® to form a solution containing 1% yeast extract and 0.025% Roxisomes®.

Normal human dermal fibroblasts were seeded into a 96 well plate at a concentration of 150,000/well and incubated at 37° C. in 5% CO<sub>2</sub> for 24 hours to enable cells to acclimate. The next day each well was treated with 200 ul of mixture with each mixture being applied to 6 wells. The plate was incubated at 37° C. for 72 hours with 5% CO<sub>2</sub>.

A 10% solution of Alamar Blue in 100 ul of DMEM was prepared. 100 µl of a 10% solution of the Alamar Blue reagent was added to each well. The plate was incubated at 37° C., 5% CO<sub>2</sub> for 2 hours. The fluorescence was read on a plate reader.

Cells were then irradiated with 10 J/cm<sup>2</sup> radiation UV Radiation Chamber (Dr. Groebel, UV-Elektronik, GmbH). The DPBS was aspirated and the treatment compositions applied once again for 24 hours. The next morning the medium was aspirated and 100 µl of 10% Alamar Blue solution was added. The plate was incubated at 37° C. for 1.5 to 2 hours.

5 The fluorescence was measured at 530/590 nanometers using a Spectra Max Gemini reader. The cell viability was calculated and expressed as the percentage of survival of cells treated with hydrogen peroxide.

	Control	1% Yeast Extract	0.025% Roxisomes	1% Yeast Extract + 0.025% Roxisomes
Average Value	2167.787	2564.93	1798.29	4393.74
Std Dev.	358.51	219.54	194.95	308.16
Pvalue	1	0.0008012	0.001132	4.60509E08
% change	0	18.32	-17.0449	102.68

10

\* expressed as a percentage increase (or decrease, if a negative number) of the untreated control cells.

The above results demonstrate that when cells are treated with the combination of the autophagy activator and DNA repair enzyme cellular viability is increased 102.68% . Thus, the combination of the autophagy and DNA repair enzyme dramatically improved cellular health and viability both before and after irradiation with UV light.

15

#### EXAMPLE 2

20

A skin treatment composition in accordance with the invention was prepared as follows:

Ingredient	w/w%
Oleth-3 phosphate	0.45
Oleth-3	0.35
Oleth-5	0.24
Butylene glycol	0.20
Squalane	0.50

BHT	0.10
Ethylhexyl methoxycinnamate	0.10
Choleth-24/ceteth-24	0.10
Triethanolamine	0.11
Retinyl palmitate/zea mays (corn) oil/BHT/BHA	0.10
Butylene glycol	1.1
Chamomile	0.03
Bisabolol	0.10
Water	QS
Methyl paraben	0.46
PEG-75	4.00
Bis-PEG-18 methyl ether dimethyl silane	2.00
Glycereth-26	1.00
Methyl gluceth-20	4.00
Trisodium EDTA	0.10
Pantethine	0.14
Caffeine	0.05
Xanthan gum	0.075
Carbomer	0.26
Triethanolamine	0.50
Phenoxyethanol	0.70
Benzyl alcohol	0.10
Bifida ferment lysate	9.40
Water/bifida ferment lysate/hydrogenated lecithin	3.00
Butylene glycol/water/ <i>Cola Acuminata</i> extract	3.00
Sodium ribonucleic acid	0.01

Water/butylene glycol/tripeptide-32*	0.20
Lactobacillus ferment/lecithin/water	0.05
Water/ <i>Arabidopsis Thaliana</i> extract/lecithin****	0.05
Phenoxyethanol	0.02
Sodium hyaluronate	0.01
FD&C Red No. 4 (1% aqueous solution with butylene glycol)	0.04
FD&C Yellow No. 5 (1% aqueous solution with butylene glycol)	0.09
D&C Green No. 5 (0.1% solution with butylene glycol)	0.001
Yeast extract**	0.001
Hydrolyzed Algin (Phyko A1, Codif Recherche & Nature)	0.001
Water	QS100

\* CLOCK or PER1 gene activator

\*\* autophagy activator

5 \*\*\* proteasome activator

\*\*\*\* DNA repair enzyme

10 The composition was prepared by combining the ingredients and mixing well to form a liquid. The composition was stored in brown glass bottles.

15 While the invention has been described in connection with the preferred embodiment, it is not intended to limit the scope of the invention to the particular form set forth but, on the contrary, it is intended to cover such alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims.

What we claim is:

1. A topical composition comprising at least one cellular autophagy activator and at least one DNA repair enzyme.
- 5 2. The composition of claim 1 wherein the cells are keratinocytes or fibroblasts.
3. The composition of claim 1 wherein autophagy activator comprises a yeast extract.
- 10 4. The composition of claim 1 wherein the autophagy activator comprises a yeast extract selected from the group consisting of those from genera *Lithothamnium*, *Melilot*, *Citrus*, *Candida*, *Lens*, *Urtica*, *Carambola*, *Momordica*, *Yarrowia*, *Plumbago*, or mixtures thereof.
- 15 5. The composition of claim 1 wherein the autophagy activator is selected from the group consisting of *Lithothamnium calcareum*, *Melilotus officinalis*, *Citrus limonum*, *Candida saitoana*, *Lens culinaria*, *Urtica dioica*, *Averrhoa carambola*, *Momordica charantia*, *Yarrowia lipolytica*, *Plumbago zeylanica*.
- 20 6. The composition of claim 1 wherein the autophagy activator is a stimulator of one or more of: (a) MAP-LC3; (b) ATG5-12; (c) protein p53; (d) AMPK; or (e) DRAM.
7. The composition of claim 5 wherein the autophagy activator is an inhibitor of mTOR.
- 25 8. The composition of claim 1 wherein the DNA repair enzyme is selected from the group consisting of base excision repair (BER) enzymes, nucleotide excision repair (NER) enzymes, DNA polymerases, DNA helicases, Mismatch repair (MMR) enzymes, and mixtures thereof.
- 30 9. The composition of claim 8 wherein the DNA repair enzyme is selected from the group consisting of *Arabidopsis Thaliana* extract either alone or in admixture with lecithin and water; *Lactobacillus* ferment, *Micrococcus* lysate, Plankton extract, Bifida ferment lysate; and mixtures thereof.
- 35 10. The composition of claim 5 wherein the autophagy activator is yeast extract is from the genus *Candida* and the DNA repair enzyme comprises *Arabidopsis thaliana* extract either alone or in admixture with lecithin and water.

11. The composition of claim 1 additionally comprising at least one proteasome activator.
12. The composition of claim 11 wherein the proteasome activator is algin, alginate, or hydrolyzed algin.

5

13. The composition of claim 1 additionally comprising a CLOCK or PER1 gene activator comprising:

10

- (a) a peptide having from about 3 to 13 amino acid residues and of the formula (I) (SEQ ID No. 1):

$R_1-(AA)_n-X_1-S-T-P-X_2-(AA)_p-R_2$ , wherein

$X_1$  represents threonine, serine, or is equal to zero,

15

$X_2$  represents an isoleucine, leucine, proline, valine, alanine, glycine, or is equal to zero,

AA represents any amino acid or derivative thereof, and n and p are whole numbers between 0 and 4,

20

$R_1$  represents the primary amine function of the N-terminal amino acid, either free or substituted by a protective grouping that may be chosen from either an acetyl group, a benzoyl group, a tosyl group, or a benzyloxycarbonyl group,

$R_2$  represents the hydroxyl group of the carboxyl function of the C-terminal amino acid, which may be substituted by a protective grouping that may be chosen from either a  $C_1$  to  $C_{20}$  alkyl chain or an  $NH_2$ ,  $NHY$ , or  $NYY$  group with Y representing a  $C_1$  to  $C_4$  alkyl chain, and

25

wherein said formula (I) sequence may contain substitutions of amino acids  $X_1$  and  $X_2$  with other chemically equivalent amino acids;

- (b) a peptide having the amino acid sequence Ser-Pro-Leu-Gln- $NH_2$ ;

30

- (c) cichoric acid; and

- (d) mixtures thereof.

35

14. The composition of claim 13 wherein the CLOCK or PER1 gene activators are selected from the group consisting of:

S -T -P - NH<sub>2</sub>

Ser-Thr-Pro-NH<sub>2</sub>

(SEQ ID No. 2) Y - V - S -T -P - Y- N- NH<sub>2</sub>

Tyr-Val-Ser-Thr-Pro-Tyr-Asn-NH<sub>2</sub>

5 (SEQ ID No. 3) NH<sub>2</sub> -V -S -T -P -E - NH<sub>2</sub>

NH<sub>2</sub>-Val-Ser-Thr-Pro-Glu-NH<sub>2</sub>

(SEQ ID No. 4) NH<sub>2</sub> -L -H -S -T- P - P - NH<sub>2</sub>

NH<sub>2</sub>-Leu-His-Ser-Thr-Pro-Pro-NH<sub>2</sub>

(SEQ ID No. 5) CH<sub>3</sub>NH -R -H -S -T -P -E - NH<sub>2</sub>

10 CH<sub>3</sub>-NH-Arg-His-Ser-Thr-Pro-Glu-NH<sub>2</sub>

(SEQ ID No. 6) CH<sub>3</sub>NH - H -S -T -P -E - CH<sub>3</sub>NH

CH<sub>3</sub>-NH-His-Ser-Thr-Pro-Glu-CH<sub>3</sub>-NH<sub>2</sub>

and mixtures thereof.

15 15. The composition of claim 13 wherein the CLOCK or PER1 gene activators are selected from:

S -T -P - NH<sub>2</sub>

Ser-Thr-Pro-NH<sub>2</sub>

(SEQ ID No. 4) NH<sub>2</sub> -L -H -S -T- P - P - NH<sub>2</sub>

20 NH<sub>2</sub>-Leu-His-Ser-Thr-Pro-Pro-NH<sub>2</sub>

and mixtures thereof.

16. The composition of claim 1 additionally comprising a CLOCK or PER1 gene activator selected from the group consisting of Tripeptide-32, Tetrapeptide-26 or mixtures thereof.

17. A method for increasing cellular viability and selective catabolysis in cells of keratin surfaces in need of such treatment comprising applying to the surface a composition comprising at least one autophagy activator and at least one DNA repair enzyme.
- 5 18. The method of claim 17 wherein the cellular viability and selective autophagy activation results in improved cellular detoxification.
19. The method of claim 17 wherein the composition is applied at night prior to evening rest.
- 10 20. The method of claim 17 wherein the keratin surfaces are skin, hair, or nails.

**A. CLASSIFICATION OF SUBJECT MATTER****A61K 8/99(2006.01)i, A61K 8/66(2006.01)i**

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 8/99; A61K 8/96; A61K 38/43; A61Q 19/00; A61K 38/08; A61Q 19/08; A61K 38/47; A61K 8/97; A61K 8/66

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) &amp; Keywords: autophagy activator, DNA repair enzyme, proteasome activator, topical, CLOCK gene activator, PER1 gene activator

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011-0243983 A1 (PAUFIQUE, J.) 06 October 2011 See claims 1, 3, 8-11.	1,3-9,11-16
A		10
Y	US 2009-0220481 A1 (MAES, D. H. et al.) 03 September 2009 See claims 1, 5-7.	1,3-9,11-16
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A	US 2011-0269694 A1 (DAL FARRA, C.) 03 November 2011 See abstract, claims 1, 3, 5, 11.	1,3-16
A	US 2003-0223982 A1 (SCHLOTSMANN, K. et al.) 04 December 2003 See abstract, claims 1, 14.	1,3-16
A	WANG, CHIEN-YU et al. 'Genomics and proteomics of immune modulatory effects of a butanol fraction of echinacea purpurea in human dendritic cells', BMC Genomics, 13 October 2008, Vol. 9, No. 479, pp. 1-30 See abstract, table 2, pages 24-27.	1,3-16

 Further documents are listed in the continuation of Box C. See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

22 April 2014 (22.04.2014)

Date of mailing of the international search report

**24 April 2014 (24.04.2014)**

Name and mailing address of the ISA/KR

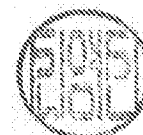
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**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: 17-20  
because they relate to subject matter not required to be searched by this Authority, namely:  
Claims 17-20 pertain to methods for treatment of the human body by therapy and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.
2.  Claims Nos.: 2  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
Claim 2 is unclear since the term 'cells' of claim 2 is not found in claim 1 to which claim 2 refers (PCT Article 6).
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

**PCT/US2013/078057**

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**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

**PCT/US2013/078057**

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