new, novel products from

Corallina officinalis alba

# Marine Moisturising Factor 1

# Snow White Coral Algae

new generation biotech products for advanced skin care formulations
INDEX

Tyrosine-Melanin reduction enzyme (s)

CAMPO MARINE MOISTURISING FACTOR 1

SPECIFICATION

Campo Snow White Coral Algae
Tyrosine-Melanin reduction enzyme(s)  
Which convert melanin in to Leuco-Melanin*

Tyosine-Melanin reduction enzymes which are responsible for the catalyst & formation of Leuco-melanin are isolated, stabilized and optimized; and are optimized bio-available from the following natural products-cosmetic functional active extracts for new novel range of skin-whitening personal-care products:

- Campo Snow White Coral Algae Extract
- Campo Pearl Extract Pws
- Campo Pearl Bezoar Acid Extract-pbaws
  * Campo Pearl Powder Extract
  * Campo Pearl Organic Germanium Extract-pogws
  * Campo Ginseng Organic Germanium Extract
  * Campo Garlic Organic Germanium Extract
- Campo Songyic Acid Complex
- Campo Songyi Gel Liquid 25% (Matsutake-Kuseki)
  * Campo Songyi Ethanol Fraction Extract and Campo Bird’s Nest Extract

*Leuco-melanin, a colorless, invisible melanin which is functional as photo-protection without darken skin pigment

Novel Structure of a Leuco-Melanin reduction catalyst Enzyme (S) as found in our Campo Novel-Skin-Whitening Active
**PRODUCT ANNOUNCEMENT**
CAMPO RESEARCH, SINGAPORE
*IN-COSMETICS 1995, PARIS*

**CAMPO MARINE MOISTURISING FACTOR 1**
*(MMF 1)*

**Campo Marine Moisturising Factor 1** is a total isolate form of *Corallina officinalis alba* (Coral Seaweed). It is tissue cultured and propagated in biotechnology vats in a controlled laboratory environment. This total extract isolate affords a number of substances resembling the water soluble natural moisturising factors (NMF) of the horny layer of human skin.

Propagation of this unique species of coral algae occurs from fragments which are broken off by wave action. It is believed that these moisturising factors participate in, or facilitate, the propagation of new individuals of the algae.

The highest content of NMF-like substances is afforded by these fragments (which are already in the process of developing into fully fledged individuals) when they are washed on to the shore and marooned on the beach during the ebb tide. It has been noted that these fragments afford the optimum concentrations of NMF-like substances.

This is believed to be due to the action of the strong Pacific sun on the broken propagating fragments which are being subjected to UV and IR radiation and heat induced dehydration. Other factors such as harsh chemical reactions may also trigger a similar response, similar to the action of soap and other anionic detergents on human skin.

A high throughput screening (HTS of the total extract, as part of a tertiary screening programme to find new AI’s, revealed a number of new bio-active compounds, in addition to other more common compounds such as amino acids, urea, fructose, niacinamide, cinnamic acid esters, etc..

![Graph 1](image-url)
The most interesting of the many novel new bio-active engineered compounds are those analogues having a structural resemblance to compounds already of interest to the cosmetics formulator:

**- COMPOUNDS IDENTIFIED IN CAMPO MMF1**

* 2-pyrrolidone-5-carboxylic acid in various salt forms including the sodium salt (Sodium PCA, as listed in CTFA Cosmetic Ingredient Dictionary, 3rd ed., 1982, p268.)

* lactates in three salt forms including the sodium salt (sodium lactate listed in CTFA Cosmetic Ingredient Dictionary, 3rd ed., p283, and DAB, p331)

* 12-epi-scalaradial - like analogue. It is known that 12-epi-scalaradial, a marine natural product, previously known only and isolated from cacospongia spp., is a potent inhibitor of bee sting and other venoms.

* Spermidine

* Spermine

* 6-methoxy-N-(3-sulphopropyl)-quinoline (SPQ)

**- KNOWN PHYSIOLOGICAL EFFECTS OF ISOLATED COMPOUNDS**

It is interesting to note that the lactate and PCA in the form of their sodium salts are well known for water bridging properties, and are widely used in cosmetics formulations for this moisturising effect.

12-epi-scalaradial acts as a potent inhibitor of toxins.

*Spermine and spermidine* are polyamines with the biological function of protection of replicating DNA against oxidative injury. Their intercellular concentration is strictly correlated to normal and pathological cell growth and protein synthesis. Spermine plays an important role in the regulation of cellular proliferation and differentiation, whilst it also facilitates regeneration of long term potentiation and protects replicating DNA against damage by singlet oxygen.

*SPQ* has an inferred biological function of minimising interference of physiological anions (and anionic detergents).

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**Graph 2**

![Graph showing moistur content over time for different conditions](image-url)
# CAMPO™ MARINE MOISTURING FACTOR 1

**SPECIFICATION:**

<table>
<thead>
<tr>
<th>Proposed CTFA Name:</th>
<th>Campo Coral Algae MMF 1 extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source:</td>
<td>Corallina officinalis alba</td>
</tr>
<tr>
<td>Appearance:</td>
<td>Liquid</td>
</tr>
<tr>
<td>Solubility (water):</td>
<td>Total dissolution / Dispersion</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH:</th>
<th>6.9 – 7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity (20 Deg. Cent.):</td>
<td>1.260 – 1.320</td>
</tr>
<tr>
<td>Refractive index (20 Deg. Cent.):</td>
<td>1.400 – 1.450</td>
</tr>
<tr>
<td>Dry residue (160 Deg. Cent.):</td>
<td>50 – 58%</td>
</tr>
<tr>
<td>Water:</td>
<td>41 – 50%</td>
</tr>
<tr>
<td>Sodium Content:</td>
<td>8 - 10%</td>
</tr>
<tr>
<td>Nitrogen Content:</td>
<td>1.8 – 2.8%</td>
</tr>
</tbody>
</table>

References:
2. DAB 8, p331
6. Merck Index, 11th ed.,
20. Illsleki, N. & Verkman, A.S., 1987, Biochemistry, 26, 1215

Campo™ Marine Moisturising Factor 1 was developed and is produced by Campo Research Pte. Ltd., of Singapore and is marketed throughout Europe and U.S.A.
**PRODUCT ANNOUNCEMENT**
CAMPO RESEARCH PTE LTD, SINGAPORE
IN-COSMETICS 1995, PARIS

**SNOW WHITE CORAL ALGAE**

**Campo Snow White Coral Algae** is a snow white pulverulent vegetal biocomposite of marine origin composed essentially of the small pink seaweed *Corallina officinalis alba*, a thermo resistant organism well known for many years as one of the marine world’s major oddities.

This small, 14cm high bush, consisting of articulated calcium rings, lives anchored in crevices hidden in rock pools along the coastlines of Samoa Island and American Samoa Island in the Pacific Ocean.

Such marine microbiotapes are particularly subject to high temperature variations. During sunny periods, temperatures are often reach in excess of 35°C within a few hours but the algae, although subjected to such thermal stress, manages to retain its cellular and metabolic integrity thanks to its unique chemical composition and skeletal structure.

Snow White Coral Algae, with its magnesium phycocalcites exceptionally rich in micronutrients presents one of the most complex structures in the algal world. A vegetal porous microbioceramic is created and formed by a labyrinth of wide cavities connected by a network of pores and galleries clinging closely to the cell walls.

The calcification phenomenon of seaweed remains today one of the most enigmatic of physiological processes. It is indeed still virtually impossible to explain why some seaweeds calcify whilst others do not. In *Snow White Coral Algae*, Carbonates are crystallised into a spectrum of rhombohedral magnesium phycocalcites, in which the Mg2+ ions, combined with various micronutrients, have been partially substituted isomorphically with calcium ions.

**SNOW WHITE CORAL ALGAE - infra-red filter**

- **Background information**

Sunlight has long been recognised as one of the most important causative factors in skin ageing, not only with respect to damage caused by UV-A and UV-B radiation, but infra-red (IR) radiation has also been implicated.

- **An effective IR filter**

Infra-red radiation is composed of wavelengths in the region 800-4000 nanometers. This thermal radiation penetrates into the cutaneous layers of the skin much deeper than UV rays, reaching successively through the epidermis
and the dermis (28-65% of initial radiation) before becoming exhausted in the hypodermis (8-17%). Infra-red radiation constitutes one of the factors responsible for accelerating the ageing process. The rays can generate severe erythemas, particularly heat erythema. Cutaneous thermal optimum value may be defined as one of the essential components to proper skin activity and integrity. Temperature variations caused by prolonged or sudden exposure to the sun, to artificial heat sources, or to cold, deviate the skin from its thermal optimum, thereby causing considerable physiological disorders. Dermal penetration of infra-red rays aggravates certain ailments such as skin blotchiness and varicose veins.

- Protection of keratinocytes against UV and IR radiation.
In-vitro studies of the protective effect of Snow White Coral Algae were carried out on a culture of human keratinocytes exposed to UV, IR and visible light for 3 hours 3 minutes. the energy received from the UV-B radiation was 2.75 J/cm².

Without protection from the Coral Algae, the keratinocyte mortality was total. At a concentration of 2%, protection of 74% of the cells was achieved, indicating good cytoprotecting power and a potential to help reduce actinic skin ageing effects.

- Protection of cell membrane
Direct exposure of skin to the sun, and hence to UV radiation, promotes the production of free radicals which have been shown, amongst other actions, to degrade cell membrane. During this exposure, membrane degradation could be worsened by oxidation reactions caused by titanium or zinc oxides currently being used widely in sun care formulations.

Unsaturated lipids are reactive molecule which are susceptible to free radical attack. They then lose their physiological functions and assist in the process of cell ageing. the first step of the degradation of unsaturated lipids in the formation of diene lipids.
- Protective effect of Snow White Coral Algae against linoleic acid oxidation created by UVB.

- Results through spectrophotometric measurement of optical density of created diene.

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>1%</th>
<th>2%</th>
<th>4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxidising effect</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>protecting effect</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Used at concentrations from 1-4%, protection of linoleic acid subjected to UVB for 30 minutes was total.

**SNOW WHITE CORAL ALGAE**

- A natural alternative to titanium and zinc oxides

Ultra-violet radiation can be conveniently divided into three ranges, UV-A, UV-B, and UV-C. The UV-A range is believed to be responsible for direct tanning of the skin without preliminary inflammation (erythema), possibly due to photo-oxidation of the leuco-form of melanin already present in the upper layers of the skin. UV-B is responsible for causing sun burn as well as for initiating reactions which lead to the formation of melanin. The production of erythema and the subsequent production of melanin reach peak at a wavelength of 296 nm.

The increase in the melanin content of the epidermis which follows exposure to UV-B, provides some degree of protection against sun burn. Granules of melanin which are formed in the basal cell layer of the skin, following the action of this radiation, migrate upwards towards the stratum corneum and the skin surface where they believed to be further oxidised by radiation in the UV-B range.

These granules are eventually shed during exfoliation of the skin, thus causing the skin to lose its protective action against further sunburn.

In the biological pathway (1) resulting in the formation of melanim i.e. melanogenesis, the precursor of melanin is the amino acid tyrosine. Melanin itself exists in two forms, *eumelanin* - a brown pigment and *pheomelanin* - a yellow pigment.
- Action of UV-A and UV-B sunblocks

The optimum particle size for highest sun protection factor (SPF) is 55-60 nm for UV-A and UV-B, and above 65-80 nm for visible sunlight filter/ sunblock protection.

There are available a wide variety of micronised titanium oxides, in both anatase and rutile forms, and zinc oxides to cover most aspects of UV-A and UV-B sunblocks actions, (table#1). Some 53 materials in the INCI list are shown as UV absorbers, plus micronised oxides and of these 24 are listed as UV filters.

Certain restrictions, however, are placed on the use of UV filters by the 76/78 EEC Directive in terms of concentration maxima, for example.

The main points to take into consideration when formulating with synthetic chemicals and micronised oxides include the pH, and the effects of the other ingredients on the SPF values. Thus, zincoxides can cause alkalinity, whilst titanium oxides are negatively charged and have a pH of around 7.4. It is difficult to produce a pH neutral titanium or zinc oxide without chemical treatment.

Particle size is particularly important factor to consider, particles below 50nm filter and protect from UV-C, whilst above 80 nm the protective action shifts to the visible region. All zinc and titanium oxides, in either anatase or rutile forms, are useless as IR filters where the wavelengths are in the region 800-4000 nm and where detrimental physiological and biological effects are different from those caused by UV light. UV light induces photochemical and photo-immunological reactions, whilst IR, in addition to causing other defects, further enhances UV damage and photo-ageing of the skin.

The structure and chemistry of Snow White Coral Algae enables it to function in two exciting ways- as a broad spectrum UV protection factor as well as a broad spectrum IR protection factor and thermal regulator.

The particle size distribution of Snow White Coral Algae brings about a broad spectrum UV protection whilst also limiting the transmission of visible light. The excessive UV rays are deflected which limits the “micro-wave oven effect” of the human skin’s molecular and atomic excitation and vibration, photochemical and photo-immunological damage and other accompanying adverse effects.

Snow White Coral Algae reduces the transmission of IR rays whilst its uniquely porous structure permits absorption of the heat generated. Absorption of IR transmission at successive layers of the skin, means that generally at the level of the dermis and epidermis transmission of some 30-

70% of the initial radiation is seen. With the addition of Coral Algae reduction to 7-12% is seen, whilst at the level of the hypodermis reduction to 0.5 - 1.0% is noted.

**Snow White Coral Algae** also acts as a thermal regulator in cold climates, greatly reducing loss of body heat and minimising the effects of local heat sources such as fires, radiators, etc., thereby balancing and maintaining normal body heat and the thermal optimum.

**Snow White Coral Algae** is a novel, natural product, with neutral pH, inert in the presence of other ingredients of sun-screen formulations, without affecting their SPF values, and does not grey in the presence of UV sunlight as is the case with most titanium oxides.

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**EFFICIENCY OF UV & IR ABSORPTION**

At 0.5%, **SNOW WHITE CORAL ALGAE** affords 85% UV and 90% IR protection. This translates into approximately 18% damaging UV rays reaching the cutaneous surface of the skin and approximately 10% IR rays reaching the epidermis. At 2% addition level, UV and IR protection reach 98% and 99% (epidermis and dermis) respectively.
COMPARISON EFFICIENCY OF BROADSPECTRUM
UV & IR REDUCTION AT 0.5% & 2% SNOW WHITE CORAL ALGA USES
SNOW-WHITE CORAL ALGAE
Corallina Offincinalis Alba

IN THE DEEP
<table>
<thead>
<tr>
<th>TRADE NAME</th>
<th>SUPPLIER</th>
<th>OXIDE</th>
<th>PARTICLE</th>
<th>FORM</th>
<th>DESCRIPTION</th>
<th>SPECIAL TREATMENT</th>
<th>PROPERTIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorosil 100TA</td>
<td>Advanced Dermaceuticals</td>
<td>Ti</td>
<td>15</td>
<td>rutile</td>
<td>pdr-surface treated</td>
<td>fluoro silicone</td>
<td>hydrophobic</td>
</tr>
<tr>
<td>Hombitec CW 5</td>
<td>Sachtleben Chemie</td>
<td>Ti</td>
<td>10-25</td>
<td>anatase</td>
<td>powder</td>
<td>lecithin</td>
<td>hydrophilic</td>
</tr>
<tr>
<td>Dermasome TO</td>
<td>Microfluidics Intl</td>
<td>Ti</td>
<td>100-200</td>
<td>undisclosed</td>
<td>liposome</td>
<td>lecithin</td>
<td>hydrophilic</td>
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<tr>
<td>Hombitec L-5</td>
<td>Sachtleben Chemie</td>
<td>Ti</td>
<td>10-25</td>
<td>anatase</td>
<td>powder</td>
<td>5% silicone oil</td>
<td>hydrophobic</td>
</tr>
<tr>
<td>Hombitec SI series</td>
<td>Sachtleben Chemie</td>
<td>Ti</td>
<td>10-25</td>
<td>anatase</td>
<td>oildispersion</td>
<td>various oils</td>
<td>hydrophilic</td>
</tr>
<tr>
<td>MT-100T</td>
<td>Tayca Corp</td>
<td>Ti</td>
<td>15</td>
<td>rutile</td>
<td>pdr-surface treated</td>
<td>aluminum stearate</td>
<td>hydrophobic</td>
</tr>
<tr>
<td>MT-100 SAS</td>
<td>Tayca Corp</td>
<td>Ti</td>
<td>15</td>
<td>anatase</td>
<td>pdr-surface treated</td>
<td>alumina/silica</td>
<td>hydrophilic</td>
</tr>
<tr>
<td>PW Covasil</td>
<td>Les Colorants Wackherr</td>
<td>Ti</td>
<td>15-40</td>
<td>anatase</td>
<td>pdr-surface treated</td>
<td>undisclosed</td>
<td>hydrophobic</td>
</tr>
<tr>
<td>Sunveil</td>
<td>Ikeda Corp</td>
<td>Ti</td>
<td>10-20</td>
<td>anatase</td>
<td>pdr-surface treated</td>
<td>tittania/silica (87/13)</td>
<td>hydrophilic</td>
</tr>
<tr>
<td>Tioveil oil based disp</td>
<td>Tioxide Specialists</td>
<td>Ti</td>
<td>40-50</td>
<td>rutile</td>
<td>aqueous dispersion</td>
<td>polydimethylsiloxane</td>
<td>hydrophobic</td>
</tr>
<tr>
<td>Tiovel AQ</td>
<td>Tioxide Specialists</td>
<td>Ti</td>
<td>40-50</td>
<td>rutile</td>
<td>aqueous dispersion</td>
<td>Al/organic treatment</td>
<td>hydrophilic</td>
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<tr>
<td>UV-Titan 262</td>
<td>Kemira OY</td>
<td>Ti</td>
<td>20</td>
<td>rutile</td>
<td>powder</td>
<td>caprylic/capric triglyceride</td>
<td>hydrophobic</td>
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<tr>
<td>UV-Titan 212</td>
<td>Kemira OY</td>
<td>Ti</td>
<td>20</td>
<td>rutile</td>
<td>powder</td>
<td>caprylic/capric triglyceride</td>
<td>hydrophobic</td>
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<tr>
<td>Spectraveil 70/MOTG</td>
<td>Tioxide Specialists</td>
<td>Zn/Ti</td>
<td>40-50</td>
<td>rutile</td>
<td>powder</td>
<td>none</td>
<td>non-selective</td>
</tr>
<tr>
<td>Spectraveil TG</td>
<td>Tioxide Specialists</td>
<td>Zn</td>
<td>40-50</td>
<td>rutile</td>
<td>dispersion in oil</td>
<td>caprylic/capric triglyceride</td>
<td>non-selective</td>
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<td>Z-cote</td>
<td>Sunsmart</td>
<td>Zn</td>
<td>10-20</td>
<td>rutile</td>
<td>powder</td>
<td>none</td>
<td>non-selective</td>
</tr>
<tr>
<td>Zinc oxide H&amp;R</td>
<td>Haarnamm &amp; Reimer</td>
<td>Zn</td>
<td>~30</td>
<td>Ti-rutile</td>
<td>powder</td>
<td>various oils / esters</td>
<td>hydrophobic</td>
</tr>
<tr>
<td>Sachtatec series</td>
<td>Sachtleben Chemie</td>
<td>Zn</td>
<td>Undisclosed</td>
<td>powder/dispersions</td>
<td></td>
<td></td>
<td></td>
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</table>
Table 2

<table>
<thead>
<tr>
<th>UV effects of the skin</th>
<th>IR effects on the skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>- photochemical disorders</td>
<td>at epidermis level</td>
</tr>
<tr>
<td>- photo immunological disorders*</td>
<td>- increases surface lipids production</td>
</tr>
<tr>
<td>- skin ageing &amp; degeneration</td>
<td>(greasy skin effect) at sebaceous and</td>
</tr>
<tr>
<td>- stimulation molecular &amp; atomic</td>
<td>epidermis level of the skin.</td>
</tr>
<tr>
<td></td>
<td>- cause typical defects in size, shape</td>
</tr>
<tr>
<td></td>
<td>and functional properties of keratin</td>
</tr>
<tr>
<td></td>
<td>cells</td>
</tr>
<tr>
<td></td>
<td>- irregular distribution of melanin in</td>
</tr>
<tr>
<td></td>
<td>epidermis cells (presence of adjacent</td>
</tr>
<tr>
<td></td>
<td>hyper-pigmented and hypo-pigmented cells</td>
</tr>
<tr>
<td></td>
<td>at dermis level</td>
</tr>
<tr>
<td></td>
<td>- increases numbers of mastocytes</td>
</tr>
<tr>
<td></td>
<td>- enhances elastic damage caused by UV</td>
</tr>
<tr>
<td></td>
<td>(formation of fine feather-like elastic fibres).</td>
</tr>
<tr>
<td></td>
<td>at hypodermis level</td>
</tr>
</tbody>
</table>

In general, IR acts in the same way as UV to accelerate the process of degeneration. IR also stimulates development of cutaneous tumours caused by UV, and can cause a severe heat rash in which the histological structure is now identified with that of actinic disease.
**TECHNICAL SPECIFICATION:**

<table>
<thead>
<tr>
<th>Technical Specification</th>
<th>Value</th>
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<tbody>
<tr>
<td><strong>CTFA name:</strong></td>
<td>algae extract</td>
</tr>
<tr>
<td><strong>CAS #</strong></td>
<td>89997-92-2</td>
</tr>
<tr>
<td><strong>EINECS#</strong></td>
<td>289-730-0</td>
</tr>
<tr>
<td><strong>Source Species:</strong></td>
<td>Corallina officinalis albas</td>
</tr>
<tr>
<td><strong>Part used:</strong></td>
<td>Thallus</td>
</tr>
<tr>
<td><strong>Appearance:</strong></td>
<td>pure White extra-fine powder</td>
</tr>
<tr>
<td><strong>Odour:</strong></td>
<td>Odourless</td>
</tr>
<tr>
<td><strong>Sieve size:</strong></td>
<td>&gt; 60-80 NM</td>
</tr>
<tr>
<td><strong>Specific surface area:</strong></td>
<td>17 cm²/cm³</td>
</tr>
<tr>
<td><strong>Porosity:</strong></td>
<td>35-60%</td>
</tr>
<tr>
<td><strong>Solvent density:</strong></td>
<td>25 cm²</td>
</tr>
<tr>
<td><strong>Solubility (water):</strong></td>
<td>Evenly disperses in water / oils, insoluble in water / organic solvents</td>
</tr>
</tbody>
</table>

**TYPICAL ANALYSIS - minerals (mg/g)**

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium</td>
<td>30.0</td>
</tr>
<tr>
<td>Sodium</td>
<td>1.00</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>10.00</td>
</tr>
<tr>
<td>Fluoride</td>
<td>0.30</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Nil</td>
</tr>
<tr>
<td>Chloride</td>
<td>1.00</td>
</tr>
<tr>
<td>Sulfate</td>
<td>0.03</td>
</tr>
<tr>
<td>Sulphur</td>
<td>2.00</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.08</td>
</tr>
<tr>
<td>Silicon</td>
<td>2.05</td>
</tr>
<tr>
<td>Preservative</td>
<td>Nil</td>
</tr>
<tr>
<td>Total germs</td>
<td>&lt;10 (Non-pathogenic)</td>
</tr>
<tr>
<td>Heavy metals:</td>
<td>0.005 ppm</td>
</tr>
<tr>
<td>Pesticides:</td>
<td>Nil</td>
</tr>
<tr>
<td>Calcium</td>
<td>80.0</td>
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</tbody>
</table>

**FORMULATION GUIDELINES:**

Addition of 0.5 - 2.0 % **Snow White Coral Algae** and 5% **Campo Pearl extract (PWS)** for an effective high SPF value sun and UV block.

**References:**


**Snow White Coral Algae, Freshwater Pearl Powder (PWS)** was developed and is produced by Campo Research Pte Ltd., of Singapore and is marketed throughout Europe and USA by:
For products of this series defined as SNOW-WHITE CORAL ALGA

These products meet the requirements for pharmaceutical preparations according to The DAB (German Pharmacopoeia) category 2:

Per 1 ml :

⇔ maximum $10^2$ aerobic micro – organisms
⇔ absence of entrobacteria
⇔ absence of Pseudomonas aeruginosa
⇔ absence of Staphylococcus aureus

The testing is carried out according to the DAB (V.2.1.8.1) and (2.1.8.2)

Additional requirements for liquid products under DAB, category 3: are also met as follows:

Per 1 ml

⇔ maximum $10^2$ yeast and moulds
⇔ absence of Escherichia coil
⇔ absence of Salmonella *

*(The testing for Salmonella is carried out with 50ml)
MATERIAL SAFETY AND CONSUMER PRODUCT
SAFETY TESTING LABS.
(DIVISION OF JTC KAMPOYAKI, SINGAPORE)
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FINAL REPORT

Sutton, Surrey,

ATTENTION: Dr. ALLAN ONIONS, Ph.D.
C. Chem.

TEST: The MATREX In Vitro
Toxicity testing System
Salmonella

typhimurium Reverse Assay

TEST ARTICLE: SNOW-WHITE CORAL
ALGA

EXPERIMENT REFERENCE NO.: 1996 - 1812

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OBJECTIVE:

To evaluate the test article for irritancy potential utilizing the MATREX in vitro toxicity testing system.

INTRODUCTION:

TESTSKIN and MATREX are sophisticated in vitro systems. Developed in Organogenesis Inc. of Cambridge, Massachusetts, they closely mimic human skin in structure and function. The Living Dermal Matrex (LDM) consist of a three-dimensional construct comprised of living cells in a collagen matrix. Nutrition is provided through the base via a permeable membrane, leaving the surface open to the atmosphere. This makes an ideal system for applying a variety of materials, including liquids, powders, oils, gels and creams.

The Living Skin Equivalent (LSE) has all the features previously described, plus the formation of an actual epidermis complete with stratum corneum.

TESTSKIN and MATREX, when used with the recommended cell metabolism assay, can quickly provide toxicological profiles. The procedure involves a solubilized, reactive tetrazolium salt (MTT), which is metabolized by the mitochondria of living cells and converted to a purple formazan dye. The color intensity of the skin replica extract, measured photometrically, correlates directly with its viability. When measured against controls, values ranging from 0% to 100% (plus or minus approximately 20%) can be calculated for each dose of an applied substance.

Test Article: CAMPO SNOW-WHITE CORAL ALGA
(10 gm evenly-dispersed in 50 ml water)

Reference Articles: PROPYLENE GLYCOL & MORPHOLINE
METHOD:

The appropriate dilutions of test sample and control articles were applied to MATREX. After the appropriate exposure period, the articles were rinsed from the MATREX surfaces. MTT (tetrazolium salt) assay medium was utilized in order to quantify cell metabolism. At the end of the staining period, excised portions of each MATREX were immersed in acidified isopropanol which extracted the converted MTT from tissue samples. A Dynatech MR 4000 Automatic Microplate Reader was used to determine the absorbance of each extract at 570 nm. With the absorbance of a negative control defined as 100%, the percent absorbancies of the test and control articles were determined. The percentages listed below directly correlate with the cell metabolism in the MATREX samples.

RESULTS:

<table>
<thead>
<tr>
<th>Test Article</th>
<th>Percent &amp; Exposure</th>
<th>Percent Viability</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNOW-WHITE CORAL ALGA (10 gm evenly-dispersed in 50ml water)</td>
<td>100% - 1 hr.</td>
<td>LDM</td>
<td>87%</td>
</tr>
<tr>
<td></td>
<td>(10% - 1 hr.)</td>
<td>LDM</td>
<td>91%</td>
</tr>
<tr>
<td></td>
<td>(1% - hr.)</td>
<td>LDM</td>
<td>94%</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>(100% - 1 hr.)</td>
<td>LDM</td>
<td>73%</td>
</tr>
<tr>
<td></td>
<td>(10% - 1 hr.)</td>
<td>LDM</td>
<td>99%</td>
</tr>
<tr>
<td></td>
<td>(1% - hr.)</td>
<td>LDM</td>
<td>96%</td>
</tr>
<tr>
<td>Morpholine</td>
<td>(100% - 1 hr.)</td>
<td>LDM</td>
<td>6%</td>
</tr>
<tr>
<td></td>
<td>(10% - 1 hr.)</td>
<td>LDM</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>(1% - 1 hr.)</td>
<td>LDM</td>
<td>100%</td>
</tr>
</tbody>
</table>

HISTORICAL IN VITRO RESULTS:
Propylene glycol has historically been categorized as virtually non-irritating when tested using the Draize irritation methodologies. Morpholine has been categorized as moderately irritating when tested in the same manner.

DISCUSSIONS:
The sponsor-submitted sample elicited in vitro results comparable to those recorded for propylene glycol.

CONCLUSION:
The results indicate that the sponsor-submitted product has virtually no irritation potential, under the conditions of this test.
CCR-Cytotest Cell Research

CCR PROJECT 95

SALMONELLA TYPHIMURIUM

REVERSE MUTATION ASSAY

REPORT

Study Completion Date:

November 23rd, 1996
Test Report CCR Project 95

CONCLUSIONS

The test article Snow-White Coral Alga was assessed for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using Salmonella typhimurium strains TA 1553, TA 1537, TA 100 and TA 102.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test article was tested at the following concentrations:

33.3; 100.0; 333.3; 1000.0; 2500.0; and 5000.0 ug/plate

No toxic effects occured in the test groups with and without metabolic activation in experiment I and II in all strains used.

The plates incubated with the test article showed normal background growth up to 5000.0 ug/plate with and without S9 mix in all strains used.

No substantial increases in revertant colony numbers of any of the five tester strains were observed following treatment with Snow-White Coral Alga at any dose level, either in the presence or absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of significance.

A slight decrease (0.001%) in revertant colony numbers was observed in strain TA 102 at 333.3 and 1000.0 ug/plate in experiment I in the presence of metabolic activation. However, this effect is considered not to be relevant since it could not be reproduced in the normally more sensitive pre-incubation assay.

Appropriate reference mutagens were used as positive controls and showed a distinct increase in induced revertant colonies.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test article did not induce point mutations by base pair changes of frameshifts in the genome of the strains used.

5 gm evenly-dispersed in 25ml of water (de-ionized)
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