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Occurence and Effects of Hormonally Active UV Filters in the Aquatic Environment

■ Introduction

Personal care products and cosmetic ingredients enter aquatic systems either directly or via effluents of wastewater treatment plants (WWTPs). UV absorbing chemicals (UV filters) are added to sunscreens and a wide variety of cosmetics (skin and hair care products, lotions, creams, fragrances). Currently, 27 UV filters are listed in the Cosmetics Directive, and a further 43 chemicals are listed as UV filters in ingredients used in cosmetics in the EU (1). In sunscreens the concentration of a specific UV filter varies between 0.5-10%, but may reach 25% (2). The amount of UV filters added is increasing, because encreasingly higher sunlight protection factors are used. Mainly two groups of UV filters are applied, often in combination; UV-absorbing organic chemicals, and inorganic ZnO and TiO_2 (nano)particles that scatter and reflect light. In addition, many organic chemicals are used as UV absorbers in textiles, household products, fabrics, plastics, optical products, agricultural chemicals and many other materials in amounts ranging between 0.05 up to 2% of the final volume, added on or into the product to protect against UV-irradiation.

Either due to direct imputs via wash-off from skin and cloth during recreational activities, or indirect pathways via wastewater or swimming pool waters, where high levels of benzophenone-3 (BP3), 4-methylbenzylidene camphor (4MBC), and 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC) of up to 40 µg/L have been detected, UV filters enter aquatic systems (3). The widespread use of BP3 in personal care products was documented in a survey in the U.S. population; 97% of 2,517 urine samples contained this compound (4). Furthermore, several UV filters were detected in human breast milk (5), demonstrating that they are accumulated in the human body.

■ Environmental Occurrence

Several UV filters were found in water, sediment and biota of aquatic systems. A summary of environmental concentrations is given in **Table 1**. Highest levels have been measured in untreated waste-

Summary

Organic UV filters as components of sunscreen-formulations directly enter the aquatic ecosystem during recreational activities. In addition their use in cosmetics and for material protection may contribute to the environmental load. In the aquatic environment UV filter residues were measured in water, sediment and organisms. At least four UV filters originating from wastewater were detected in Swiss rivers in the following order of decreasing concentrations: benzophenone-4 (BP4) > benzophenone-3 (BP3) > 3-(4-methylbenzylidene-camphor) (4MBC) > 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC). UV filters accumulate in biota, and recently up to 337 ng/g lipid of EHMC was detected in fish. It was found that most UV filters exhibit hormonal activity *in vitro*. In fish benzophenone-1, benzphenonon-2 (BP2), 3-benzylidene camphor (3BC) and ethyl-4-aminobenzoate (Et-PABA) lead to a significant induction of vitellogenin, which serves as a biomarker for estrogenicity in fish. Furthermore, exposure to 3BC and BP2 caused feminization of the secondary sex characteristics in male fish, alteration of gonads in male and female fish and a decrease in fertility and reproduction. The lowest observed effect concentrations for 3BC and BP2 were 3 µg/L and 1.2 mg/L, respectively. The estrogenic effect of UV filter mixtures was additive to antagonistic in fish. This indicates that a risk assessment of UV filters in the aquatic environment should be based on mixture activity.

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Environmental Sample	UV Filter	Max. Conc. (ng/L, mg/kg)	Location	Reference
Lake water	4MBC	80	Switzerland	(14)
	BP3	125		
	ED-PABA	34		
	EHMC	92	Slovenia	(11)
	OC	31		
	BM-DBM	24		
	HBP	85	Korea	(15)
River water	HMS	345	Slovenia	(11)
	BP3	114		
	EHMC	88		
	OC	34		
	4MBC	17	Switzerland	(17)
	DHB	47	Korea	(15)
Seawater (beach)	4MBC	799	Norway	(13)
	BP3	440		
	EHMC	390		
	OC	7301		
Raw drinking water	EHMC	5610	California	(13)
Raw wastewater	4MBC	6500	Switzerland	(20)
	BP3	7800		
	EHMC	19000		
	OC	12000		
Treated wastewater	4MBC	2700	Switzerland	(20)
	BP3	700		
	EHMC	100		
	OC	270		
Swimming pool water	4MBC	330	Slovenia	(11)
	BP3	400		
Fish (lakes)	4MBC	3.80 mg/kg (lw)	Germany	(19)
	HMS	3.10 mg/kg (lw)		
	EHMC	0.31 mg/kg (lw)		
	BP3	0.30 mg/kg (lw)		
	4MBC	0.17 mg/kg (lw)	Switzerland	(20)
	BP3	0.12 mg/kg (lw)		
	EHMC	0.07 mg/kg (lw)		
	OC	0.02 mg/kg (lw)		
Fish (rivers)	4MBC	0.42 mg/kg (lw) [1]	Switzerland	(20)
	OC	0.63 mg/kg (lw) [1]		
	EHMC	0.34 mg/kg (lw)	Switzerland	(17)
	BP3	0.09 mg/kg (lw)		
Cormorants	EHMC	0.70 mg/kg (lw)	Switzerland	(17)
Sewage sludge	4MBC	1.78 mg/kg (dm) [1]	Switzerland	(9)
	EHMC	0.11 mg/kg (dm) [1]		
	OC	4.84 mg/kg (dm) [1]		
	OTC	5.51 mg/kg (dm) [1]		

[1] mean concentrations. , lw: lipid weight, dm: dry matter

Table 1 Maximal concentrations of UV filters in the aquatic environment and biota

water and lower levels in treated wastewater, indicating removal to various degrees (6, 7). In untreated municipal wastewater up to 19 µg/L EHMC (6), 10.4 µg/L BP3, 2.1 µg/L 4MBC in China (7), and 1.5 µg/L benzophenone-4 BP4 (8) have been detected. Lower levels of 0.06-2.7 µg/L 4MBC, 0.01-0.7 BP3, 0.01-0.1 EHMC and 0.01-0.27 octocrylene (OC) occurred in treated wastewater in Switzerland (6), and 1.36 µg/L BP4 in Spain (8). UV filters are accumulated in digested sewage sludge (9), and sediments (10, 11). Moreover, UV filters are found in landfill leachates (12) and they are released from coatings from building parts (9).

UV filter residues were detected in lakes, rivers and beaches of coastal areas, where highest levels of 390 ng/L EHMC 440 ng/L BP3, and 799 ng/L 4MBC, and 7301 ng /L OC have been reported (13).

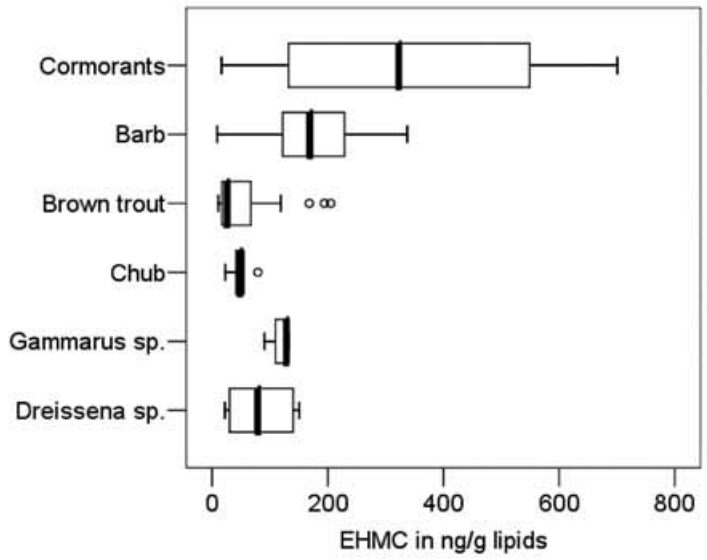

Fig. 1 Concentrations of EHMC (as box-whisker plots) in 50 biological samples of species of different trophical levels in rivers in Switzerland. ○ = outlier (adapted from 17)

Compound	Estrogenic activity	Antiestrogenic activity	Androgenic activity	Antiandrogenid activity
4-Methylbenzylidene camphor (4MBC)	--	+++	--	+++
3-Benzylidene camphor (3BC)	+	+++	--	+++
Benzophenone-1 (BP1)	+++	--	--	+++
Benzophenone-2 (BP2)	+++	--	+++	+++
4-Hydroxy benzophenone (HBP)	+++	--	--	+++
4,4'-Dihydroxybenzophenone (4DHB)	+++	--	--	+++
Benzophenone-3 (BP3)	+	+++	--	+++
Benzophenone-4 (BP4)	+	+++	--	+++
Isopentyl-4-methoxycinnamate (IMC)	--	+++	++	+++
Ethyl hexyll methoxycinnamate (EHMC)	--	+++	++	+++
Octocrylene (OC)	--	+++	+	+++
Benzyl salicylate (BS)	+	+++	--	+++
Phenly salicylate (PS)	++	+++	--	+++
Homosalate (HMS)	--	+++	+++	+++
Octyl salicylate (OS)	--	+++	++	+++
Para amino-benzoic acid (PABA)	--	+++	--	--
Ethyl-4 amino benzoate (Et-PABA)	+++	--	--	++
Octyl dimethyl para amino benzoate (OD-PABA)	--	+++	--	+++
Ethoxylated ethyl 4-amino benzoate (Peg25-PABA)	--	+	--	--

+++, maximal dose-response curves with ≥ 80% efficacy; ++, submaximal dose-response curves with ≥ 30% efficacy; +, submaximal dose-response curves with < 30% efficacy. Bold, most potent hormonal activity found for each compound; --, not detected.

Table 2 Hormonal activities of UV filters *in vitro* in the recombinant hERα and hAR assay. After (23)

Up to 125 ng/L BP3 were detected in lakes in Switzerland (14), 345 ng/L homosalate (HMS) in Slovenia (11), 85 ng/L 4-hydroxybenzophenone and 47 ng/L of 2,4-dihydroxybenzophenone in Korea (15). Polar UV-filters such as benzophenone-1 (BP1) (15) and BP4 (8) were also detected in rivers. In the river Glatt (Switzerland) we detected the four UV filters with the following order of decreasing concentrations BP4 > BP3 > 4MBC > EHMC (16, 17). River water concentrations of BP3, 4MBC and EHMC were between 6-68 ng/L. Wastewater was the most important source. Surprisingly, even at very remote environments such as the Pacific Ocean (near Polynesia) EHMC, 4MBC, BP3 and 3-benzylidene-camphor (3BC) occurred in the surface microlayer (18).

UV filters are also accumulated in biota. The presence of lipophilic UV-filters (4MBC, EHMC, OC, BP3, HMS) was reported in fish with lipid-weight concentrations of up to 3100 ng/g in Germany (19), 1800 ng/g 4MBC and 2400 ng/g OC in rivers (20), and 166 ng/g 4MBC in lakes in Switzerland (6). Recently, we studied the occurrence of UV filters in different aquatic species of different trophic levels. EHMC was prevalent in all samples at concentrations of up to 337 ng/g lipids (16, 17) and was prevalent in all aquatic invertebrates (mussels, Gammarus), fish, and in cormorants **(Fig. 1)**. In some samples, BP3 was detected in addition to EHMC.

Hormonal Effects *In Vitro*

Using the yeast reporter gene system YES (21), we have shown that nine of 18 analysed UV filters and one metabolite exhibited estrogenic activity by activation of human estrogen receptor α (hERα). Similarly, estrogenicity was observed in a recombinant yeast system expressing the rainbow trout estrogen receptor alpha (rtERα) (22). Moreover, a high proportion of commonly used UV filters has been found to exhibit multiple hormonal activities in vitro including estrogenicity, antiestrogenicity, androgenicity and antiandrogenicity through interactions with hERα and/or human androgen receptor (hAR) (23). As much as 14 UV filters exhibited antiestrogenicity, and a high number antiandrogenic activity **(Table 2)**. The majority of UV filters displayed as much as three distinct hormonal activities each.

Hormonal Activity of UV Filters *In Vivo* and Effects on Fertility and Reproduction in Fish

Aquatic organisms are continuously exposed to UV filters in wastewater contaminated environments. We investigated the *in vivo* estrogenic activity after exposure of juvenile fish (fathead minnows) for 14 days to nine different UV filters and determined the induction of vitellogenin (VTG), which serves as a biomarker for estrogenicity in fish. Ethyl-4-aminobenzoate (Et-PABA) (23), 3BC, BP1 and BP2 led to significant VTG induction (22). 3BC showed the highest potency with a significant VTG induction at 435 µg/L and higher **(Fig. 2)**. The lowest observed effect concentrations for estrogenicity of Et-PABA, BP1 and BP2 were consider-

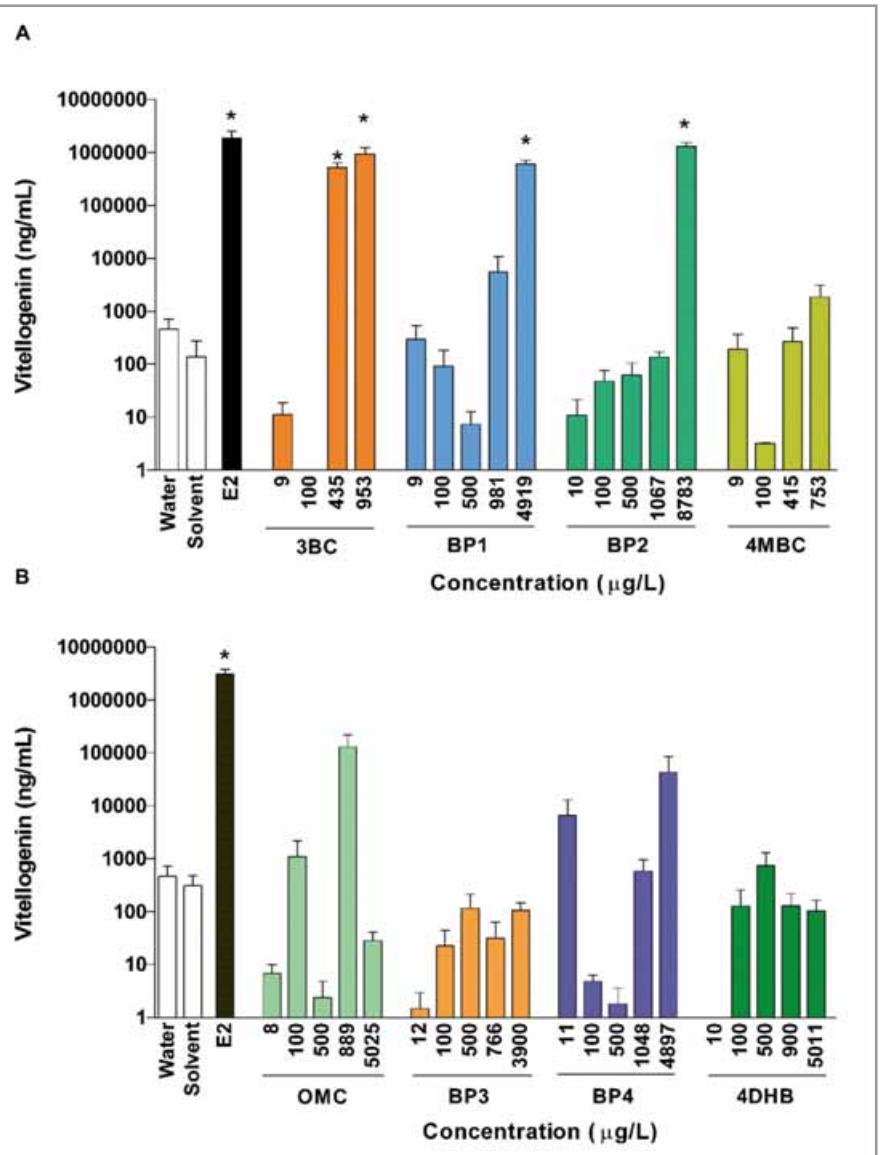

Fig. 2 Vitellogenin concentration in juvenile fathead minnows exposed to eight UV filters. Values are means ± SEM (n=10). Asterisk denotes a significant difference from control (solvent) at $p \leq 0.05$. Concentrations given as actual median measured, except 100 and 500 µg/L. Estradiol (100 ng/L) served as positive control (adapted from 23)

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ably higher. The UV filters 4MBC, OMC, BP3, BP4, 4DHB did not induce VTG in fathead minnows up to the highest concentrations tested. However, estrogenicity was found at 620 µg/L BP3 in juvenile medaka (24). The induction of VTG and choriogenin mRNA in male medaka liver occurred after a 7 days exposure of medaka at 9.9 mg/L EHMC and 99 mg/L 4MBC (25).

We investigated 3BC as the most potent estrogenic UV filter for possible adverse effects on fertility and reproduction in fathead minnows (26). An exposure for 21 days lead to dose-dependent VTG induction and reduction of male secondary sex characteristics, but also to the reduction of spawning activity due to histological alterations in the gonads. Significant VTG induction in male fathead minnows was observed at 74 µg/L 3BC and higher, but gonadal alterations were observed already at 3 µg/L. In males a significant and dose-related decrease in the number of tubercles was observed **(Fig. 3)**. Males at the highest exposure concentration were visually not discernible from females and all but one had lost all tubercles. The development of nuptial tubercles and a fatpad in the head region in male fathead minnows is stimulated by testosterone. Their atrophy in 3BC-exposed males may have resulted from an inhibition of testosterone production by 3BC.

3BC significantly affected reproductive output of pair-breeding fathead minnows (26). Females stopped reproducing immediately after the onset of exposure at a concentration of 74 µg/L 3BC and higher. The cumulative number of eggs spawned decreased significantly and gonad histology was altered. The lowest observed effect concentration for the most sensitive parameter, gonad histology, was 3 µg/L. At 74 and 285 µg/L oocyte and spermatocyte development was inhibited in male and female gonads **(Fig. 4)**. Testes of exposed males had much fewer spermatogenic cysts, and ovaries of exposed females had much fewer mature and more atretic follicles. At 74 and 285 µg/L females stopped egg production and the release of mature oocytes. This is possibly a reaction to the missing mating behavior of demasculinized males.

Similarly, exposure of fathead minnows breeding pairs to BP2 resulted in a dose-dependent demasculinisation of males, indicated by the loss of secondary sexual characteristics, significant VTG induction and the inhibition of spermatogenesis (27). Dose-dependent VTG induction and a decrease in the number of nuptial tubercles was observed from 0.1 mg/L BP2 onwards. Reproduction was also nega-

Fig. 3 Disappearance of nuptial tuberles in male fathead minnows as a demasculinizing effect of 3BC. A) Control fish. B) Fish exposed to 74 µg/L 3BC. C) Fish exposed to 285 µg/L 3BC (according to (26)

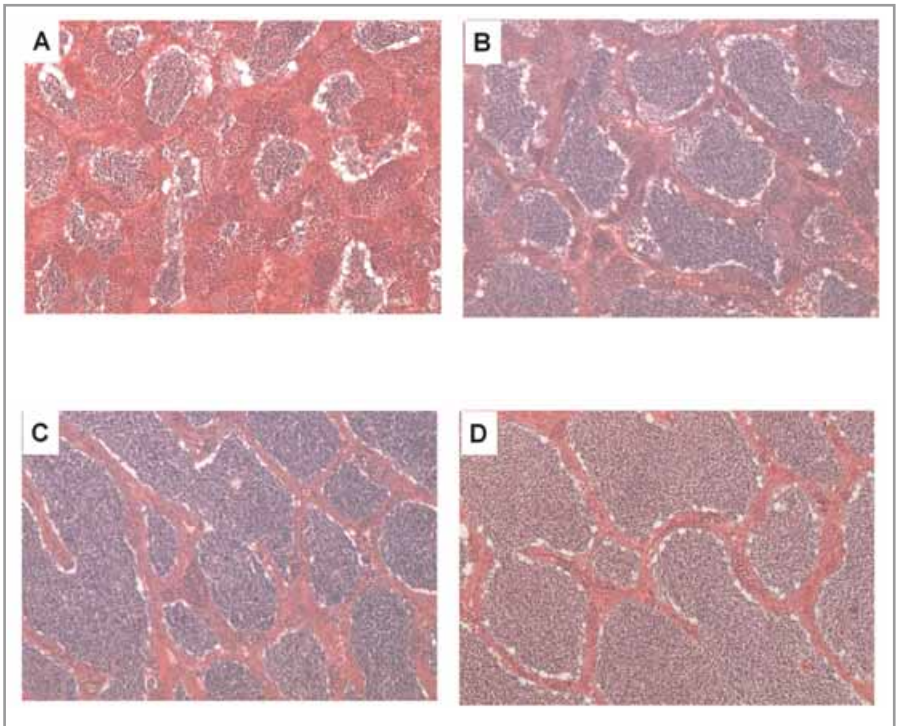

Fig. 4 (A) Section of testis from a control spawing male. (B) Testis from fish exposed to 3 (B), 33 (C) and 285 µg/L (D) 3BC. Note the enlarged seminiferous tubules filled with sperms and relative lack of germinal epithelium in the tubules. (from (26))

tively affected in a dose-dependent manner. At 1.2 mg/L BP2, fish showed reduced spawning activity. Females exposed to 5.0 mg/L and 9.7 mg/L BP-2 stopped spawning immediately after the onset of exposure. Similar histological alterations in testes and ovaries indicating inhibition of spermatogenesis and oogenesis occurred as previously observed for 3BC. At concentrations of 1.2 mg/L BP2 and higher, oocyte development was significantly inhibited.

Effects of UV Filter Mixtures

UV filters are often applied as mixtures in sunscreens. In the environment, they also occur as mixtures. We found that most mixture show a synergistic interaction *in vitro* (28). In order to better understand interactions of UV filters regarding their effects on fish, we investigated three commonly used UV filters, for their estrogenic mixture activity and analyzed their joint effects by using the concentration addition (CA) concept and nonlinear isobolograms (29). The estrogenic UV filters 3BC, BP1 and BP2 act as pure- or partial estrogen receptor agonists *in vitro*. We exposed juvenile fathead minnows for 14 days to six concentrations of each UV filter alone to determine VTG induction curves, calculate equi-effective mixture concentrations and predict mixture effects. For 3BC, BP1 and BP2 significant VTG-induction occurred at 420, 2'668, and 4'715 µg/L, respectively **(Fig. 5)**. BP2 displayed a full dose-response curve, whereas 3BC and BP1 showed submaximal activity of 70 and 78%, respectively. Subsequently we exposed fish to 6 equi-effective mixtures (EC-NOEC, EC1, EC5, EC10, EC20, EC30) of these UV filters. Significant VTG induction occurred at EC5 and higher **(Fig. 5)**. The curves for the observed and predicted mixture activity agreed for mixture levels (EC10 to EC30), demonstrating additive interaction, however, at EC-NOEC, EC1 and EC5, a lower activity was observed than predicted by CA. Detailed isobolographic analysis indicate additivity at EC10 to EC30, and antagonism at low levels (EC-NOEC to EC5). Our data show that the activity of UV filter mixtures are additive to antagonistic, indicating that the joint action of UV filters should be taken into account for their hazard and risk assessment.

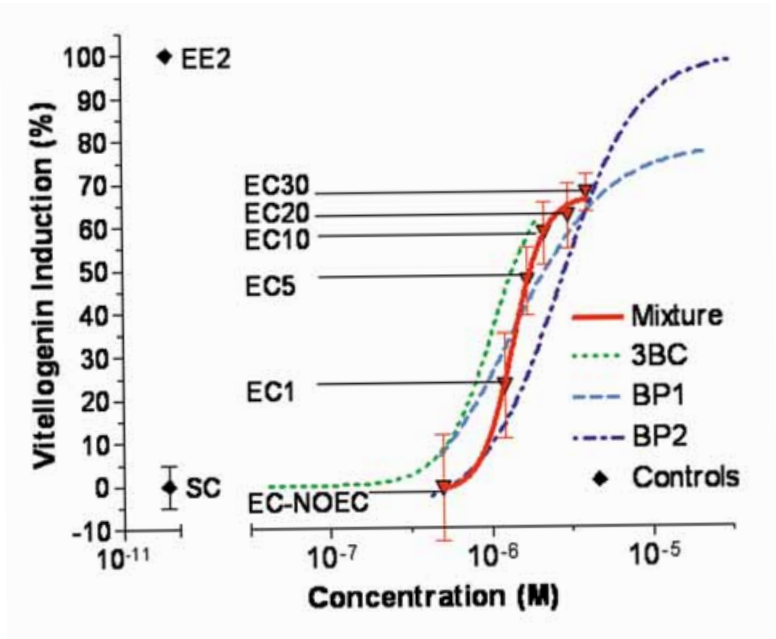

Fig. 5 Vitellogenin dose-response curve for equi-effective ternary mixture of 3BC, BP1 and BP2 at 6 different effect levels. Data are shown as means ± SEM (three replicates with six fish each). Dotted lines represent dose–response curves of single mixture compounds (blue), and mixture activity curve is shown in red (adapted from 29)

Environmental Hazard and Risk Assessment

An environmental risk assessment (ERA) can be based on the ratio of measured environmental concentrations of a given chemical in the environment and the predicted no observed effect concentrations (PNEC), which is based on ecotoxicological data taking a safety factor into account (30). Accodingly, we performed an ERA for UV filters based on the current knowledge.

Highest MEC of UV filters were 238 ng/L (EHMC), 268 ng/L (BP3), and 488 ng/L 4MBC detecte at beaches (13). We found up to 17 ng/L 4MBC, 68 ng/L BP3 and 6 ng/L EHMC in the river Glatt (17), and 849 ng/L BP4 were determined in other rivers (8). UV filters also occur in fish (20). The ecotoxicological effects data to estimate PNECs of BP3, BP4, 4MBC and EHMC are based on VTG induction in fish (22-24) and acute toxicity in Daphnia magna (31). No significant vitellogenin induction was measured for BP3 (12 µg/L to 3900 µg/L), BP4 (11 µg/L to 4897 µg/L), 4MBC (9 µg/L to 753 µg/L) and EHMC (8 µg/L to 5025 µg/L) in fish (22). In contrast, estrogenic activity and reproduction effects have been observed in medaka for BP3 at 0.62 mg/L (24). Induction of VTG mRNA was detected at 9.87 mg/L EHMC and 9.92 mg/L 4MBC (25). Consequently, the no observed effect concentrations (NOEC) for EHMC and 4MBC were taken as 9.9 mg/L.

Table 3 shows that the risk quotient (MEC/PNEC) for BP3 and BP4 are low. Based on current data, a tentative ERA indicates that the risks on aquatic biota are low for these UV filters, when considered as single compounds (30). The risks of 4MBC and EHMC are unclear. It should be emphasised, however, that the ERA is based on acute toxicity in Daphnia, on estrogenic activity and effects on reproduction in fish. We cannot rule out that other so far unknow effects may occur at even lower concentrations. Forthcoming experiments will focus on the

UV-filter	Acute toxicity Daphnia	Chronic (VTG, reprod.) fisch(mg/L)	NOEC or LOEC (mg/L)	Safety factor	PNEC (µg/L)	MEC (µg/L)	MEC/PNEC	Environmental Risk
BP4	50[1)]	–	–	1000	50	0.849[5)]	0.02	NO
BP3	1.9[1)]	0.62[2)]	0.62	100	6	0.440[6)]	0.07	NO
4MBC	0.56[1)]	9.92[3)]	–	1000	0.56/9.9	0.799[6)]	1.43/0.08	UNCLEAR
EHMC	0.29[1)]	9.87[3)]		1000	0.29/9.9	0.390[6)]	1.35/0.04	UNCLEAR
3BC	–	0.003[4)]	0.003[4]	100	0.03	0.009[7)]	0.3	NO

[1)] (31) [2)] (24) [3)] (25) [4)] (26) [5)] (8) [6)] (13) [7)] (18)

Table 3 Environmental risk assessment of single UV filters based on measured environmental concentrations (MEC) and effects in fish and Daphnia magna. Safety factors of 1000 were taken for acute data in Daphnia and 100 for chronic data in fish (BP3, 3BC).

mechanisms of action of UV filters using gene expression analysis. Our studies also demonstrate that mixtures of 3BC, BP1 and BP2 caused antagonistic interactions at low, and additivity at higher concentrations in fish (29). As UV filters actually occur in mixtures in the aquatic environment mixture activity has to be taken into account in an ERA. Therefore, it cannot be ruled out that a mixture of UV filters may pose an ecotoxicological risk to aquatic organisms.

■ Conclusions

Many UV filters are ubiquitous in aquatic systems and lipophilic compounds such as EHMC is accumulated in biota. Ecotoxicological studies demonstrated that some UV filters show estrogenic activity in fish. 3BC and BP2 significantly affected fertility and reproduction. Based on our current knowledge, the ERA indicates that the risk for the single UV filters to fish and Daphnia is low or unclear. Given the few ecotoxicological endpoints measured, this ERA is tentative, as undiscovered effects may well occur. UV filters occur as mixtures in the environment and their effects on the hormone system may sum up to levels, where a potential risk cannot be ruled out.

Acknowledgement

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UV-Induced Premature Senescence as a Model to Study Photo-Aging: A Case Study with 1,25 Dihydroxyvitamin D3

Introduction

It is known that many proliferative cell types like lung or skin human diploid fibroblasts (HDFs), melanocytes, endothelial cells, retinal pigment epithelial cells, erythroleukemia cells, exposed to subcytotoxic stress (UV, organic peroxides, H_2O_2, ethanol, mitomycin C, hyperoxia, etc.), undergo stress-induced premature senescence or SIPS which is closely related to replicative senescence (1, 2). SIPS can be defined as the long term effects of subcytotoxic stress on proliferative cell types, including irreversible growth arrest of a majority of the cell population. SIPS is characterized by several biomarkers that are common to cells having reached the limit of their proliferative life span. In particular, the proportion of HDFs positive for senescence-associated β-galactosidase (SA β-gal) activity increases in SIPS (3). This activity is attributed to an increased expression of the lysosomal β-D-galactosidase protein (4). Cellular senescence is also characterized by cell-growth arrest, morphological changes, ROS overproduction (which could explain the maintenance of a senescent state) or gene expression changes.

UVB-induced SIPS represent valuable *in vitro* model to evaluate the efficacy of anti-ageing compounds against the skin ageing processes induced by chronic sun exposure (photoageing).

1,25 dihydroxyvitamin D3 (calcitriol), the active metabolite of vitamin D, is well known for its function in bone metabolism, but cannot be restricted to this. Indeed, its role in photoprotection and immunosupression following UV exposure has been demonstrated in keratinocytes culture (5).

This article was undertaken in order to determine whether 1,25 dihydroxyvitamin D3 is able to help skin cells to repair damages caused by external stresses such as those induced by UV light exposure.

It was shown that the level of appearance of several senescence-associated biomarkers, induced by UVB in human BJ foreskin fibroblasts, strongly decreased by a pre-treatment with calcitriol. The results also support the proof-of-concept that UVB-induced premature senescence can be used in skin photoageing research.

Materials and Methods

1,25 dihydroxyvitamin D3 (calcitriol) was purchased from sigma and used at the concentration of 0,22 µg/ml in 0,05% ethanol.

Abstract

Fighting senescence and the appearance of age signs is of particular interest in a cosmetic point of view. Skin is submitted to intrinsic ageing, linked to genetic characteristics of individuals (chronological ageing) and to extrinsic ageing. Extrinsic ageing is linked to environmental exposure to stress which is mainly represented by UV-exposure. UV are responsible for the appearance of the ageing, by inducing DNA damage, radical oxygen species production, and matrix remodeling.

An *in vitro* model of UV-induced premature senescence of human dermal fibroblasts has been developed, presenting senescence markers, characteristic of photo-ageing. Repeated short non toxic UV-exposures allow cells to reach a senescent phenotype, closely related to replicative ageing. This model has been recognized to evaluate and demonstrate anti-senescence activities of cosmetic ingredients.

This article describes a clear protection after pre-treatment of cells with the active metabolite of vitamine D3, the 1,25 dihydroxyvitamin-D3 (calcitriol) against UV-induced premature senescence. Using gene expression arrays, some interesting insights and pathways are also identified that may be involved in the protective effect.

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Cell culture, exposure to UVB and detection of SA-β-galactosidase
BJ foreskin HDF (ATCC) at early cumulative population doublings were cultured in DMEM (Invitrogen, UK) containing 1% fetal calf serum (FCS, Invitrogen, UK). BJ HDFs at 50-60% of confluence were submitted to four repeated subcytotoxic exposures to UVB at 125 mJ/cm² with the BIOSUN (Vilber Lourmat), with one stress per day, for 4 days, as described in **Fig. 1**.

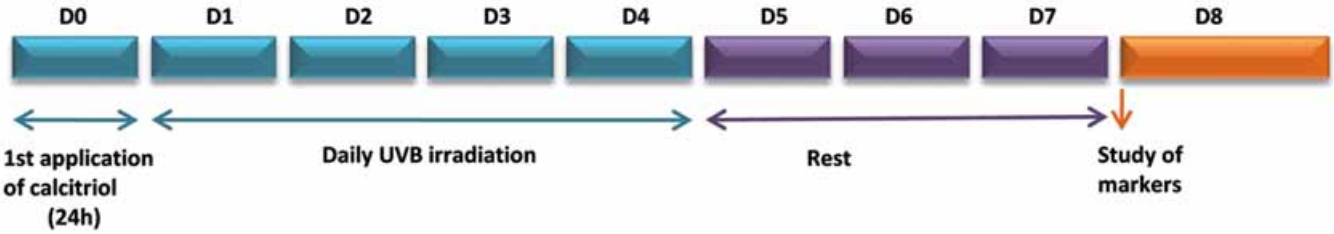

Fig. 1 Schematical procedure of UVB exposure and recovery period (SIPS-UVB model) of human dermal fibroblasts

Untreated control cultures followed the same schedule of medium changes without UVB treatment. For a 24h pre-incubation period and following every stress, the culture medium was replaced by medium containing 0,22 µg/ml calcitriol. After the last UVB exposure, cells were incubated in the presence of calcitriol for 72 hours. At the end of the recovery time, the SA-β-gal activity of the cells was assessed by colorimetry (4). A phosphate-buffered saline solution was used to cover cells during the UVB exposures, in order to discard any filtering effect from the molecule.

Detection of DNA damage (pyrimidine dimers formation)
Cyclobutane pyrimidine dimers (CPDs) were detected by immunofluorescence using the TDM-2 monoclonal antibody, according to the manufacturer's instructions (Medical and Biological Laboratories Ltd, Japan), after the 72h of recovery period.

Cell proliferation assay
After the recovery period, cell proliferation was assessed by BrdU incorporation. BJ HDFs were seeded in a 96-well plate. BrdU Proliferation measurement was performed using the Cell Proliferation ELISA, BrdU (Roche). This colorimetric immunoassay is designed for the quantification of cell proliferation, based on the measurement of BrdU incorporation during DNA synthesis. Measurement of absorbance allows the assessment of proliferation relative to controls.
The effect on cell proliferation was also assessed on fibroblasts and keratinocytes (Lonza, Belgium), without UV exposure. In brief, cells were seeded in 96-well plate and calcitriol was applied for 24h and 72h on cell culture. BrdU proliferation assay was then performed. The controls for these experiments were mitomycin (0,5 ng/ml), bFGF (5 ng/ml) and KGF (20 ng/ml).

ROS production
The oxidant-sensitive probe H2DCFDA (2', 7'-dichlorodihydrofluorescein diacetate, Molecular Probes) was used to determine the intracellular levels of ROS in cells.
72 hours after the last stress, cells were seeded in 24-well plates containing 1 ml of DMEM 10% FCS. The day after, cells were washed with PBS and incubated for 1 hour in the same buffer containing 5 µM H2DCFDA dissolved in ethanol. After washing, the fluorescence intensity was measured using a micro-plate fluorescence reader.

Gene expression study using the »DualChip Human Ageing«
The DualChip™ Human Aging has been designed to efficiently target stress and aging-related gene expression patterns comprising apoptosis, growth factors, extracellular matrix, DNA damage, oxidative stress, cell signaling, cell proliferation, DNA repair, transcription, apoptosis and inflammation (Eppendorf, Germany). The array contains 240 genes, including housekeeping genes and internal controls of the entire process (6).
Total RNA was extracted with the RNeasy Mini Kit (Qiagen) and its concentration was determined by spectrophotometry. Reverse Transcription was performed with a biotin-dNTP mixture. cDNA was then hybridized on the DualChip Human Aging. After staining, the array was scanned using the Eppendorf SilverQuant scanner and following SilverQuant software recommendations. The SilverQuant Analysis software is then used for spot finding and quantification. Array images were further quantified and normalized.

Results

Calcitriol protects human BJ foreskin fibroblasts against senescence-associated beta-glactosidase activity

To determine whether 1,25 dihydroxy vitamin D3 protects against UVB-induced senescence, BJ HDFs were treated by repeated UVB exposures, leading to premature ageing. 0,22 µg/ml calcitriol was included in the culture medium, 24 hours before the stress, following every stress and during the 72-hours recovery phase. At the end of the recovery time, the percentage of SA-β-gal positive cells was measured by colorimetry. As illustrated in **Fig. 2**, the percentage of cells expressing SA β-gal in HDFs after repeated UV exposures was dramatically reduced in the presence of calcitriol, reaching a value even lower to that of the non-irradiated controls.

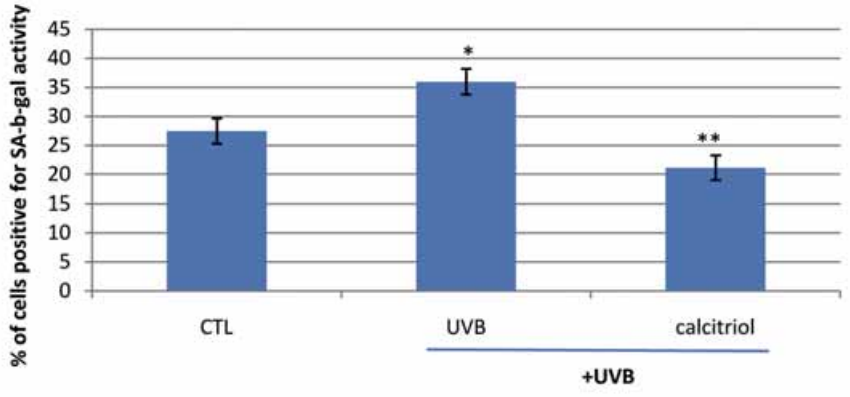

Fig. 2 Percentage of SA-β-galactosidase positive cells after induction of SIPS by repeated UVB exposure. After staining as described (4), 400 cells were counted per well and the number of positive cells for SA-β-gal staining was evaluated. Triplicates were performed

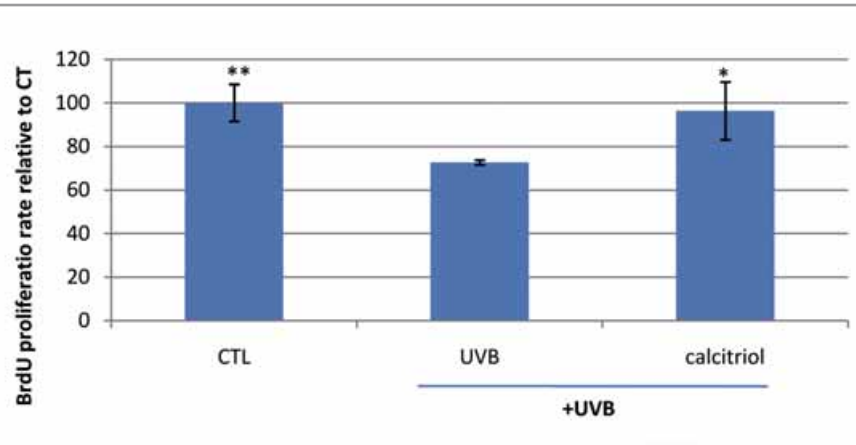

Fig. 3a Cell proliferation rate after 72 h recovery period of the UVB-induced SIPS, evaluated by BrdU incorporation. Results are reported to the control, arbitrarily set to 100%. Histograms represent the mean of three independent experiments with their relative standard deviation

Effect of calcitriol on skin fibroblasts proliferation

The influence of calcitriol on BJ HDFs proliferation was tested using a BrdU incorporation assay. The results in **Fig. 3** demonstrate that calcitriol significantly increased cellular proliferation as compared to UVB-treated cells, restoring a cell proliferation rate similar to control, unexposed cell.

The direct effect of calcitriol on cell proliferation was also evaluated after application in the culture medium of fibroblasts or primary keratinocytes (data not shown) for 24h or 72h. The results showed in **Fig. 4** demonstrate that cellular proliferation is significantly decreased by calcitriol.

Effect of calcitriol on ROS production

After 72h of recovery after the last UV exposure, ROS production was measured by the fluorescent probe H2DCFDA. Results showed in **Fig. 5** indicate a strong decrease of ROS production when cells are treated with calcitriol.

Effect of calcitriol on DNA damage repair after UVB exposure

We tested whether calcitriol could prevent the appearence of UVB-induced senescence by protecting the cells against DNA damages. When the cells were pre-incubated with calcitriol, 24h before UVB exposure and after each exposure, immunofluoresence labeling of CPDs showed that calcitriol seems to decrease the appearance of CPD, as compared to UVB treated cells, without calcitriol **(Fig. 6)**.

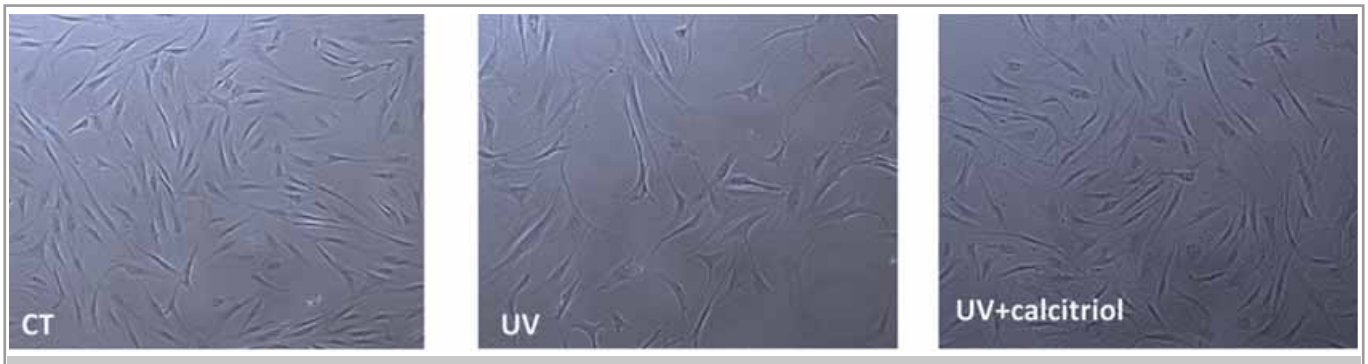

Fig. 3b Pictures taken at the end of the recovery period of UVB-induced-SIPS. Cell morphology and number of control (CT) and cells submitted to UVB exposure pretreated with calcitriol are similar, while in the UVB exposed cells condition, cell number is decreased. Arrows indicates senescent cells, flattened and enlarged

Gene expression analysis
Using the low-density array »DualChip™ Human Aging«, the influence of calcitriol on gene expression after induction of SIPS-UVB was studied. Results are presented in **Table 1**.

■ Conclusion

SIPS, or cellular senescence induced prematurely by exposure to sub-cytotoxic H_2O_2 or repeated UVB, is characterized by the appearance of several biomarkers, common to the replicative senescence. Among others, cell cycle arrest is observed as well as SA-β-gal activity or the constant overproduction of reactive oxygen species (ROS). The effect of calcitriol was studied in UVB-induced SIPS of human dermal fibroblasts, on the appearance of these biomarkers, after its application before the first UVB exposure, after each exposure and during the recovery period.
Calcitriol, the active metabolite of vitamine D_3 is already known for its photoprotective role *in vitro* on human keratinocytes (7). It was shown that calcitriol has also an effect on the protection against appearance of senescence induced by UVB exposure of human dermal fibroblasts. Indeed, it prevents the appearance of SA-β-gal activity, a well-known marker of senescence.
Senescent cells are also characterized by cell growth arrest. The effect of calcitriol on cell proliferation is very interesting. Indeed, without stress, application of calcitriol induced cell growth arrest, while when added during SIPS, it prevents the cell proliferation arrest. This could probably be explained by a global effect on senescence prevention. Calcitriol is known to act in a biphasic way on keratinocytes proliferation *in vitro*. Indeed, at high concentration, it decreases proliferation, while, at a lower concentration, it increases proliferation (8). Moreover, the results obtained demonstrate that calcitriol doesn't prevent cellular senescence by increasing cell proliferation of non-senescent cells.
Calcitriol also prevents the endogenous overproduction of ROS, which is suspected to be responsible of the maintenance of the senescent state. Indeed, when dermal fibroblasts are submitted to stress, such as UV exposure, ROS are overproduced. The major intracellular ROS intermediate is hydrogen peroxide. It was shown that calcitriol treatments prevented this overproduction, protecting cells against further damage and sustained endogenous metabolic stress.
A preliminary experiment showed its protective effect against the appearance of specific UV-induced CPDs, confirming results obtained by (9) that demonstrate that pre-treatment of keratinocytes with calcitriol prevents DNA damage induced by UV exposure. These preliminary results showed that calcitriol prevents appear-

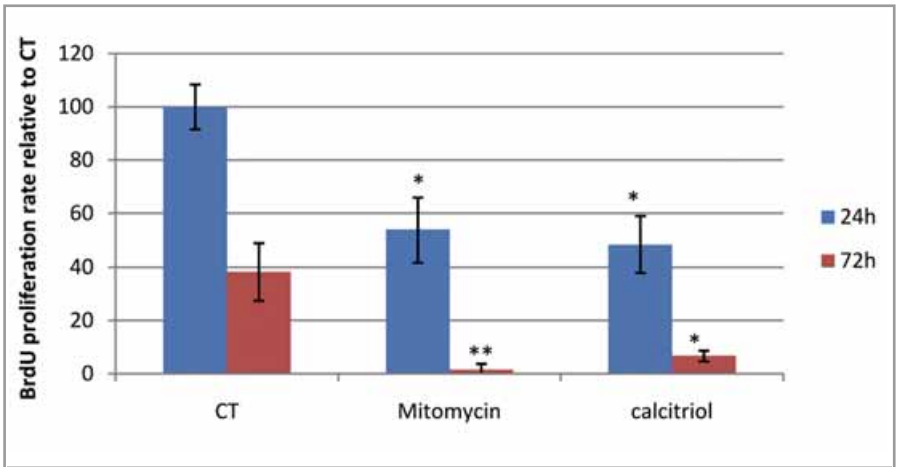

Fig. 4 Fibroblasts proliferation rate evaluated by BrdU incorporation. Results are reported to the control, arbitrarily set to 100%

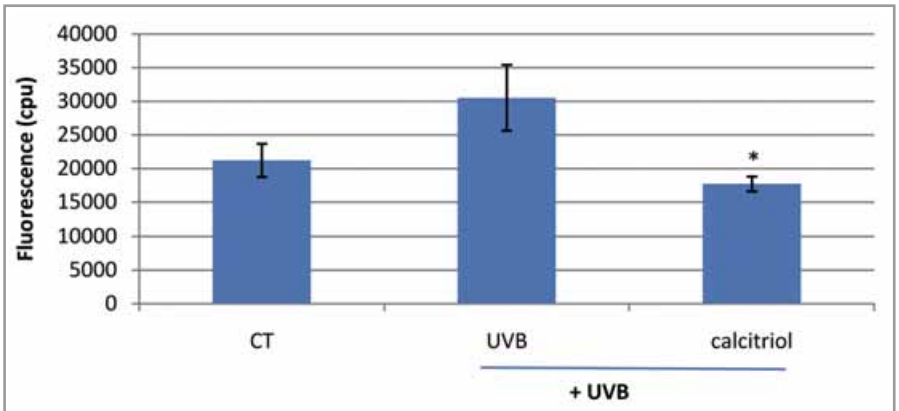

Fig. 5 ROS production evaluated by fluorescence (DCFDA) detection after UVB induced SIPS. Histograms present the results of triplicates

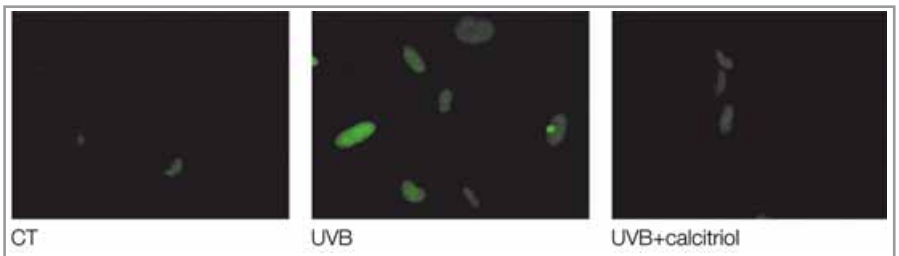

Fig. 6 Pictures taken at the end of the recovery period of UVB-induced-SIPS. Immunofluorescence labeling of CPDs was performed

Table 1 Results of gene expression changes from hybridization on Dual Chip Human aging low density array. Results are expressed as increase or decrease to control untreated test

Gene name	Gene ID	General Function	Norm. Ratio
Enhancer of polycomb homolog 2 (Drosophila)	EPC2	/	1,5145
Amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)	APBB1	Alzheimer's disease	1,5552
Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	PTGS1	Arachidonic acid metabolism	3,0992
Keratin 19	KRT19	Cell Communication	-1,5047
Collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	COL3A1	Cell Communication / Focal adhesion / ECM-receptor interaction	2,0493
Collagen, type VI, alpha 2	COL6A2	Cell Communication / Focal adhesion / ECM-receptor interaction	1,838
Thrombospondin 1	THBS1	Cell Communication / TGF-beta signaling pathway / Focal adhesion / ECM-receptor interaction	-1,9019
Cyclin B1	CCNB1	Cell cycle	2,6879
Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	CDKN1B	Cell cycle	1,6738
E2F transcription factor 1	E2F1	Cell cycle	2,1744
Proliferating cell nuclear antigen	PCNA	Cell cycle	1,6485
Polo-like kinase 1 (Drosophila)	PLK1	Cell cycle	4,2469
Retinoblastoma 1 (including osteosarcoma)	RB1	Cell cycle	1,7762
Cyclin-dependent kinase 4	CDK4	Cell cycle / Tight junction / T cell receptor signaling pathway	1,5125
Collagen, type XV, alpha 1	COL15A1	Cell differentiation	1,4975

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Gene name	Gene ID	General Function	Norm. Ratio
Histone 1, H4i	HIST1H4I	Chromosomal processing	2,4634
Kinesin family member 23	KIF23	Chromosomal processing	3,0136
Nuclear receptor subfamily1, group D, member 1	NR1D1	Circadian rhythm	1,5811
Plasminogen activator tissue	PLAT	Complement and coagulation cascades	1,7446
Plasminogen activator,Urokinase	PLAU	Complement and coagulation cascades	1,5233
Tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	TNFRSF11B	Cytokine-cytokine receptor interaction	-1,9444
Interleukin 11	IL11	Cytokine-cytokine receptor interaction / Jak-STAT signaling pathway / Hematopoietic cell lineage	2,0776
Poly (ADP-ribose) polymerase family, member 1	PARP1	DNA repair / synthesis	1,6376
Topoisomerase (DNA) II alpha 170kDa	TOP2A	DNA repair / synthesis	2,5339
Ubiquitin-conjugating enzyme E2 variant1	UBE2V1	DNA repair / synthesis	-1,4969
Matrix metallopeptidase 11 (stromelysin 3)	MMP11	Extracellular matrix	-2,5255
Matrix metallopeptidase 3 (stromelysin 1, progelatinase)	MMP3	Extracellular matrix	-4,7008
Insulin like growth factor 1 receptor	IGF1R	Focal adhesion / Adherens junction	1,6364
Insulin-like growth factor binding protein 2, 36kDa	IGFBP2	Growth factor and cytokines	1,6755
V-raf-1 murine leukemia viral oncogene homolog 1	RAF1	MAPK signaling pathway / Dorso-ventral axis formation / Focal adhesion / Gap junction / Natural killer cell mediated cytotoxicity / Long-term potentiation / Long-term depression / Regulation of actin cytoskeleton / Insulin signaling pathway	1,5035
Dihydrofolate reductase	DHFR	One carbon pool by folate / Folate biosynthesis	2,2105
Glucose-6-phosphate dehydrogenase	G6PD	Pentose phosphate pathway / Glutathione metabolism	1,6668
Heme oxygenase (decycling) 1	HMOX1	Porphyrin and chlorophyll metabolism	1,8179
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 11	PSMD11	Proteasome	1,5185
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 12	PSMD12	Proteasome	1,5631
Eukaryotic translation initiation factor 3, subunit 6 48kDa	EIF3S6	Protein biosynthesis	1,5971
HLA-B associated transcript 1	BAT1	Protein metabolism	1,5512
HtrA serine peptidase 1	HTRA1	Proteolysis	2,6521
Ribonucleotide-reductase M1 polypeptide	RRM1	Purine metabolism / Pyrimidine metabolism	2,0955
Polymerase (DNA directed), alpha 2 (70kD subunit)	POLA2	Purine metabolism / Pyrimidine metabolism / DNA polymerase	3,2895
Thymidine kinase 1, soluble	TK1	Pyrimidine metabolism	1,709
Stomatin (EPB72)-like 2	STOML2	Receptor binding	1,7596
Sorcin	SRI	Signal transduction	1,5427
Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	ID2	TGF-beta signaling pathway	3,6731
Early growth response 1	EGR1	Transcription factor activity	1,994
V-myb myeloblastosis viral oncogene homolog (avian)-like 2	MYBL2	Transcription factor activity	2,3506
Fibromodulin	FMOD	Transforming growth factor beta receptor complex assembly	2,101
Ubiquitin-conjugating enzyme E2C	UBE2C	Ubiquitin mediated proteolysis	2,0697

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ance of CPDs, in a UVB-induced senescence model of dermal fibroblast, confirming its photoprotective action.
The use of a specific, stress and ageing-focused low-density DNA array allowed us to identify potential mechanistic pathways of protection, such as the activation of the proteasome, the surexpression of genes involved in anti-oxidant mechanism or the decrease of metalloprotease synthesis, as well as increase of collagen expression.
These results indicate that calcitriol could be considered *in vitro* as a photo-protective agent acting against cellular senescence. Use of safe mimetic vitamin D3 molecules in cosmetic ingredients could be considered as promising approach to slow the appearance of senescence as a result of photo and chronological ageing.

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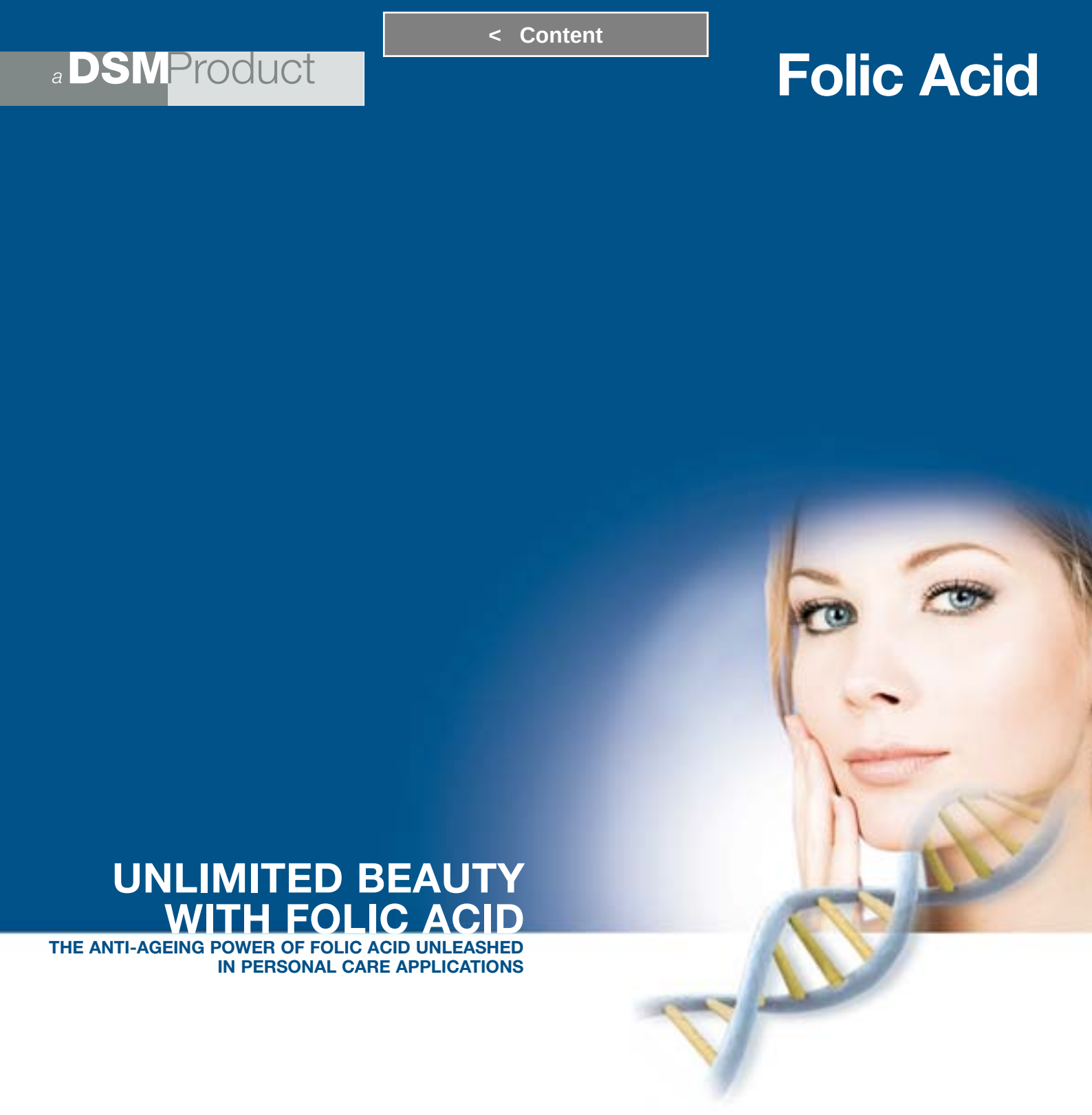

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M. Mateu*, M. Mangues*, J. Cebrián*, C. Carreño**, N. Almiñana*

A New Accelerator and Long-Lasting Tanning Patented Peptide

Introduction

In the 20th century a new trend started which has grown throughout the years until our days: the tanning culture. Nowadays, people spend hours under the sun or in the tanning salons to get their longed sun-kissed skin as tanned skin has become a symbol of health and beauty.

The genetically constitutive skin colour and tan is determined by the skin level of the melanin biopolymer pigment. The constitutive pigmentation determines to a large extent the skin pigmentary response to ultraviolet (UV) radiation to burn or tan, which has been used to classify skin phototypes (types I–VI) (1).

UV-stimulation of skin pigmentation over the basal constitutive level, commonly called tanning, involves an increase in the melanocyte activity and a stimulation of melanin neosynthesis and melanocyte dendricity, a crucial morphological feature required for melanin transfer to keratinocytes (2).

Depending on the UV exposure time, tanning proceeds in three steps: immediate pigment darkening (IPD), persistent pigment darkening (PPD) and delayed tanning (DT) response. However, the only one resulting from the stimulation of melanin synthesis is DT, which also involves an increase in the tyrosinase activity and in functional melanocytes, dendricity, synthesis and transfer, as well as altered packaging of melanosomes (1).

Skin pigmentation is the most important natural photoprotective factor present in the skin, as melanin acts as a broadband UV absorbent and presents antioxidant and radical scavenging properties. Melanin synthesis takes place in melanosomes, which are contained in specialised cells, called melanocytes, placed in the basal layer of epidermis (2). Melanosomes development involves matrix organisation, followed by formation and deposition of melanin until melanosomes are fully melanised (3). Melanin-containing melanosomes migrate towards the extremities of the melanocyte dendrites where they are transferred to the surrounding keratinocytes, ensuring a uniform distribution of melanin pigments in epidermis.

UV radiation induces the expression of the proopiomelanocortin (POMC) and melanocortin 1 receptor (MC1R) genes, which encode the α-melanocyte-stimulating hormone (α-MSH) and its receptor, respectively, which play a crucial role in pigmentation (4). MC1R is a seven transmembrane domain G-protein-coupled receptor that binds the agonists α-MSH and adrenocorticotropic hormone

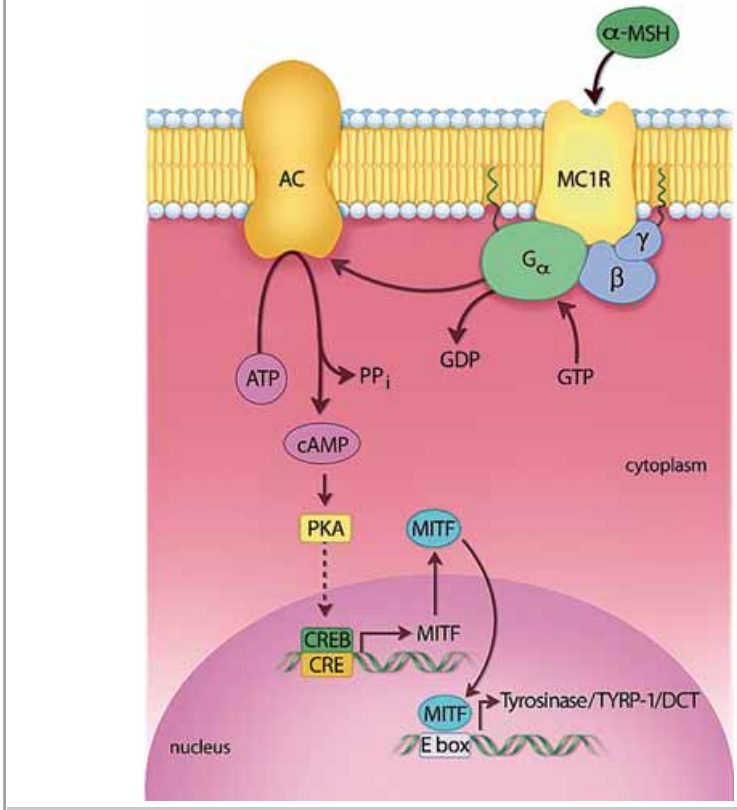

Fig. 1 Melanogenic pathway via cAMP

(ACTH) that stimulate the synthesis of melanin.
Once MC1R is activated, Gα dissociates from Gβ and γ subunits and stimulates the intracellular messenger adenylyl cyclase (AC) which, in turn, catalyses the conversion of cytoplasmic adenosine triphosphatase (ATP) to cyclic adenosine-3',5'-monophosphate (cAMP) (5). Inside the cell, increased intracellular cAMP acts as the second messenger binding to the two sites of the regulatory subunits of protein kinase A (PKA), allowing the catalytic subunits to be liberated and activated. PKA is a serine/threonine kinase inactive tetramer consisting of two regulatory subunits and two catalytic subunits (6). PKA translocates to the nucleus where activates the expression of microphthalmia-associated transcription factor (MITF).

MITF is a basic helix-loop-helix melanocyte-specific transcription factor that regulates transcription of genes encoding tyrosinase and TYRP-1 (tyrosine-related protein) and DCT (dopachrome tautomerase, also called TYRP-2). Tyrosinase is a binuclear copper-containing enzyme regarded as the key enzyme in melanogenesis being absolutely necessary for pigmentation. cAMP can influence the transcription of tyrosinase via MITF and its central role in control of tyrosinase expression has been widely demonstrated (7). TYRP-1 and DCT are melanogenesis related proteins which also respond in a cAMP-dependent manner via MITF **(Fig. 1)**.
Tyrosinase mediates the first and rate-limiting step of melanin formation. Tyrosinase along with TYRP-1 and TYRP-2 are the enzymes responsible for the conversion of tyrosine in eumelanin or pheomelanin in presence of a sulphydryl donor (glutathione or cysteine), which are the two major types of melanin pigments in mammals.
The capacity of melanocytes to synthesise melanin varies enormously. Melanocytes in dark skins or with the capacity to tan well have a great capacity to synthesise melanin and transfer it onto the surrounding keratinocytes, while in fair skin their capacity is very limited (8).
Each melanocyte produces both eumelanin (black or brown) and pheomelanin (red or yellow) but the ratio varies greatly and determines the hue of the skin. Dark skin mostly have eumelanin but also smaller amounts of pheomelanin, whilst fair skin present small amounts of melanin in the epidermis with large

quantities of pheomelanin and a less amount of eumelanin.
The main used active ingredient in commercial self-tanning sunless formulations is dihydroxyacetone (DHA). DHA and other self-tanning agents used in combination with DHA such as erythrulose, glyceraldehyde or hydroxymethylglyoxal are reducing sugars which react with free amino groups of the stratum corneum and the epidermis through the Maillard reaction to give melanoidins, which are responsible for the brown colour, and other Advanced Glycation End products (AGEs). AGEs can result in stiffening, photoaging, induce oxidative stress, lipid peroxidation products and protein cross-linking. In order to avoid a pronounced photoaging process, sun exposure should be avoided after the application of self-tanning products containing reducing sugars (9).
A new patented peptide has shown to possess both tanning accelerating and long-lasting properties via the skin melanogenesis pathway. Melatime™ (proposed INCI name: Palmitoyl Tripeptide-30) has proved *in vitro* its hyperpigmenting activity by increasing cAMP levels that stimulate melanin synthesis. Unlike reducing sugars, from which resulting tan comes together with AGEs, the peptide increases the own melanin content, providing the skin with a natural tan that fits its constitutive colour. In addition, as melanin presents antioxidant, radical scavenging and UV scattering and absorbent properties, the fact to increase melanin content implies also that the skin receives an extra protection which can help to prevent photoaging signs.
Melatime™ is also regarded as a tanning accelerator, as it proved *in vivo* to provide a perfect sun-kissed skin in less time. In consequence, UV-exposure times shorten and also the free radicals effects derived from the radiation exposition. Furthermore, the peptide also prolonged the tan after two weeks since the last irradiation, showing to possess a long-lasting effect.

■ Materials and Methods

cAMP levels determination

The *in vitro* test to determine cAMP levels is based on the competition between free cAMP and cAMP Tracer (cAMP-acetylcholinesterase conjugate) for a limited number of cAMP-specific antibody binding sites. The product of the enzymatic reaction between the substrate and the cAMP Tracer has a distinct yellow colour which can be determined spectrophotometrically, giving the proportional amount of cAMP Tracer bound to the well. Absorbance was read at 405 nm and values were normalised in respect to the control.
Cellular cAMP levels were analysed in a G361 human caucasian melanocyte cell line, in presence of vehicle, 10 µM Melatime™ or 40 µM forskolin. Vehicle was used as negative control and forskolin, which is known to increase cAMP levels and to cause a progressive and robust darkening associated with dose-dependent accumulation of melanin in epidermic cells, was used as positive control.

Melanin quantitation

G361 human melanocyte cells were cultured and incubated for 4 days with Melatime™ at 10 µM, 50 µM and 100 µM. After treatments, cells were trypsinised and melanin was extracted and quantitated. Absorbance was measured at 470 nm in a spectrophotometer, and values were normalised in respect to the number of cells per well. Melanin concentration was determined in pg/cell from a standard curve plotted with synthetic melanin at known concentrations in the same solvent. Vehicle was used as a negative control.

Evaluation of melanin accumulation and melanin content determination in primary human melanocytes

Primary human epidermal melanocyte cells were seeded and incubated with Melatime™ at 10 and 100 µM in complete medium until confluence was reached. Vehicle was used as negative control, and 40 µM forskolin as positive control. Photographs were taken with a microscope using a 10X lens after 4 days of treatment.
Melanin content was calculated from images using Image-Pro Plus image analysis software (Media Cybernetics, Inc). Brightness, contrast and gamma adjustments were applied as necessary. Colour density was specifically used to trace and count melanin areas automatically. The percentage of tanning was expressed as colour density of melanin signals.

In vivo test: Long term evaluation of skin colorimetry after UVA irradiation (preliminary test)

5 healthy caucasian female and male volunteers, aged between 25 to 35, from phototypes II, III, IV (according to Fitz-Patrick) applied a cream containing 5% Melatime™ Solution (0.0005% Melatime™) on one forearm and placebo cream on the other, once a day for 4 weeks. Both forearms were exposed to UVA irradiation, three times a week during the first 2 weeks, under controlled conditions. The UVA dose was chosen between 8 and 25 J/cm² on the basis of the individual MPD (Minimal Pigmenting Dose) and the light source was placed directly in contact with the skin of the subject's forearm.
The instrumental evaluation of skin colorimetry, which was performed by means of a Chromameter CR-400, and digital images of the forearms were taken at the beginning and after 7 and 28 days of treatment (two weeks after the last irradiation).
The parameters L*, a* and b* were measured; where L* is luminance which represents relative brightness, from L*=0 (total darkness) to L*=100 (absolute white), a* is the red/green colour axis and b* is the yellow-blue colour axis.
The best description of a brightening effect is given by combining the L* and b* parameters, in the Individual Typological Angle ITA°, which is obtained according to the formula:

$$ITA^\circ = \text{Arctg}\,[(L^*-50)/b^*]\cdot(180/\pi)$$

The darker is the skin, the lower are the L* and ITA° parameters.

■ Results and Discussion

cAMP levels determination

Determination of cAMP levels in G361 human melanocyte cells for 24 hours showed that Melatime™ was able to increase cAMP production by 56.7% **(Fig. 2)**.

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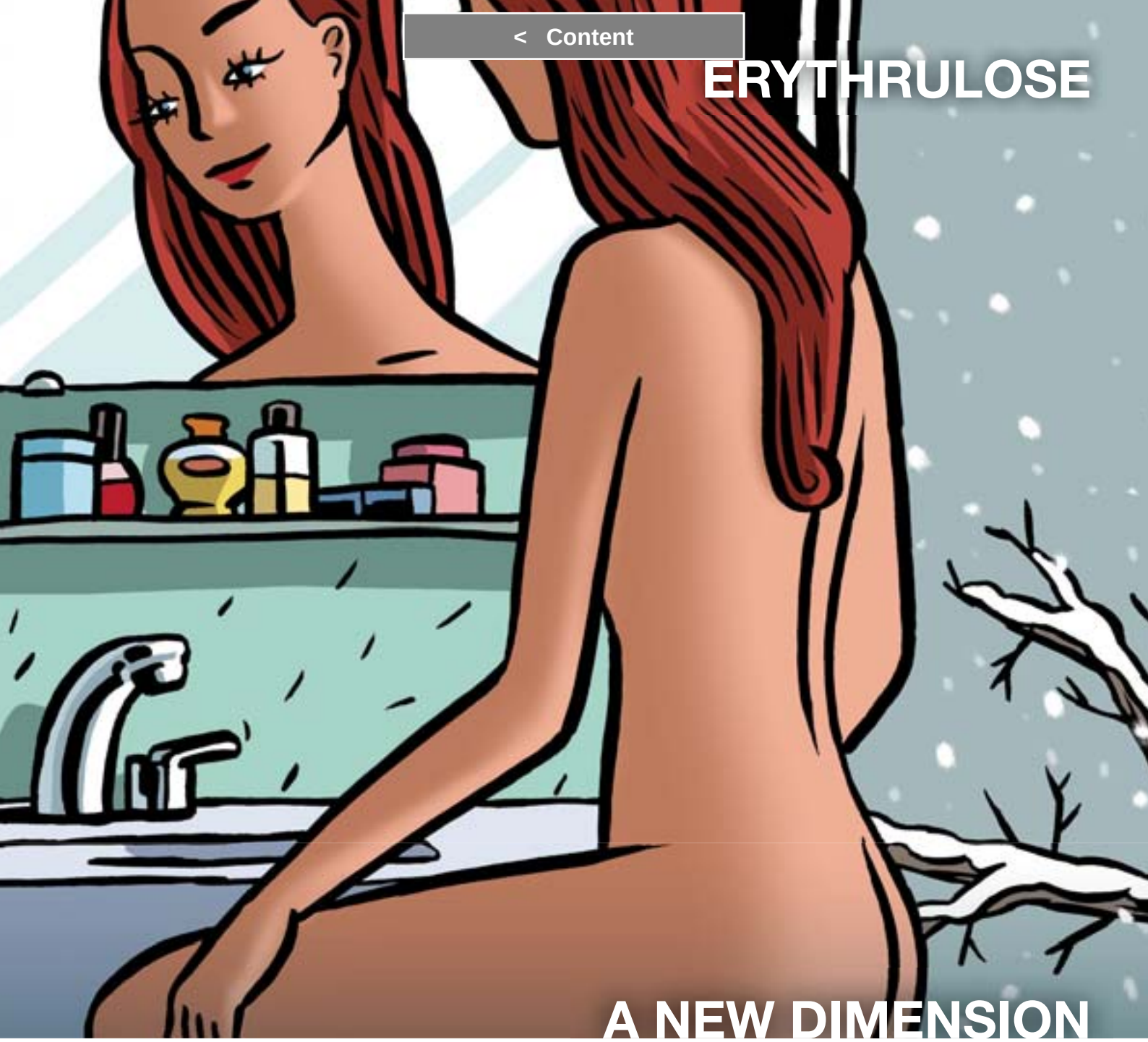

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Melanin quantitation
The results obtained for the melanogenic activity clearly manifested that Melatime™ was able to stimulate melanin synthesis in cultured G361 human melanocyte cells in a dose-response manner, reaching values of 138% induction at the higher dose tested **(Fig. 3)**.

Evaluation of melanin accumulation and melanin content determination in primary human melanocytes
Average percentages obtained on melanin accumulation from the image analysis of human epidermal melanocyte cells exposed to Melatime™ showed that the peptide increases melanin production in a dose-dependent manner, increasing melanin content in 180% after 4 days of treatment **(Fig. 4)**.
Melatime™ induced melanogenesis in the melanocytes, increasing the content of melanin **(Fig. 5)**.

***In vivo* test: Long term evaluation of skin colorimetry after UVA irradiation (preliminary test)**
There was a decrease in the L* and ITA° values of Melatime™ at all the control times, meaning that there was a tanning acceleration of the skin colour during the UVA irradiation period and a long-lasting effect 14 days after the last irradiation.
The peptide decreased by 57.5% and 108.6% the L* and ITA° values, respectively, compared to placebo in the first week of treatment under UVA-inducing conditions, showing more efficacy in accelerating the tanning. The reduction of the L* and ITA° values in presence of Melatime™ after 28 days of treatment (14 days after the last UVA irradiation) was of 48.1% and 40.4% respectively compared to placebo, proving to be more effective in prolonging the suntan **(Fig. 6)**.
Under UV irradiation conditions, Melatime™ accelerated the tanning process **(Fig. 7)**, with respect to the area treated with the placebo.

■ Conclusion

A new patented tanning peptide has proved to act as a tanning accelerator and long-lasting key active ingredient for tanning formulations by stimulating the skin natural tanning mechanisms. Melatime™ showed to induce cAMP production and stimulate melanogenesis in melanocytes, increasing melanin content in a dose-response manner in human melanocytes in absence of UV radiation, proving its efficacy in vitro as self-tanning agent and ensuring a skin nat-

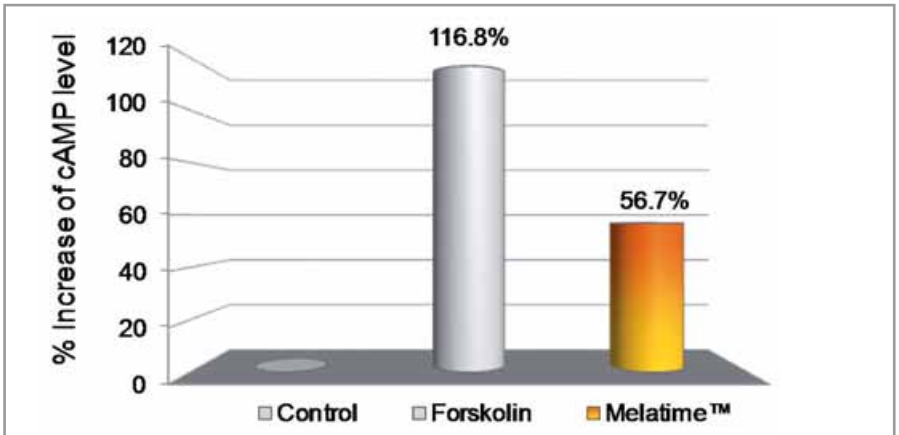

Fig. 2 Percentage of cAMP levels increase on G361human melanocyte cell line

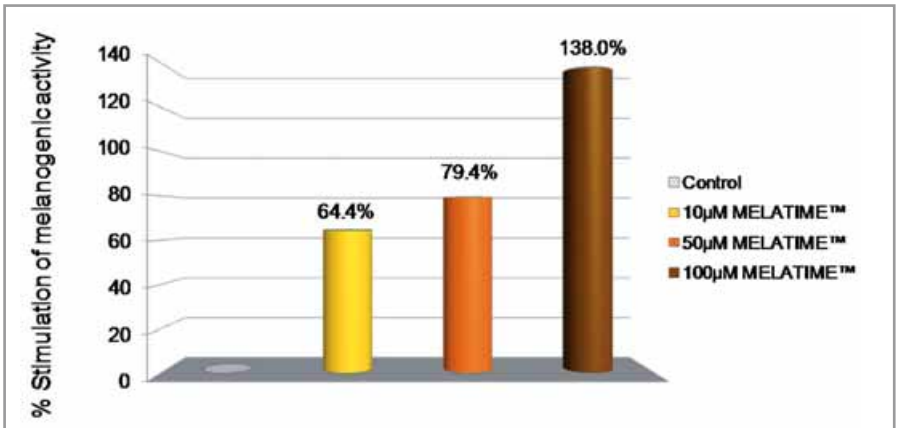

Fig. 3 Dose response assays of the melanogenic activity of Melatime™ at different concentrations on G361 human melanocyte cells

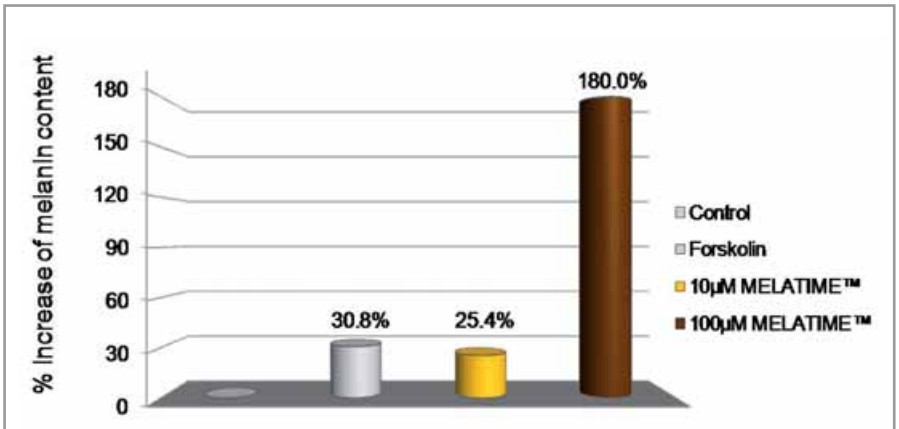

Fig. 4 Percentage of melanin production after 4 days of treatment with Melatime™ at different concentrations

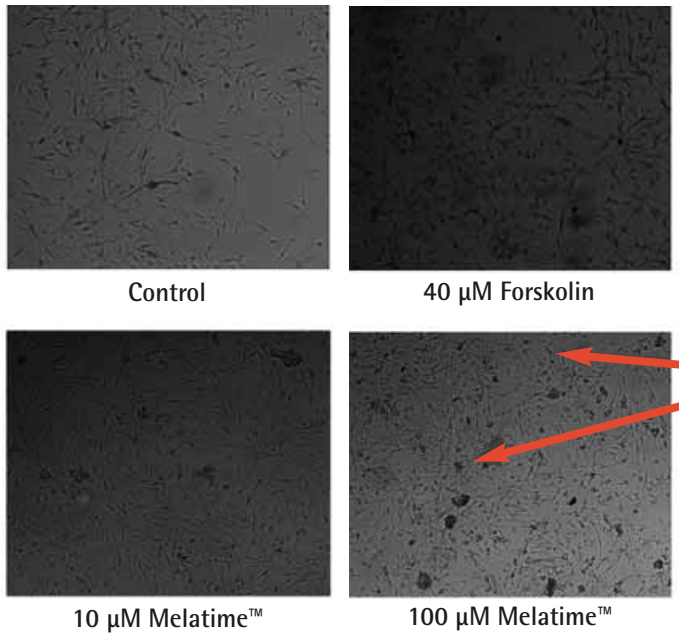

Fig. 5 Photographs of human epidermal melanocyte cells treated with Melatime™ after 4 days of treatment

ural tan which fits the skin constitutive colour.

Under UV-induced conditions, Melatime™ decreased skin luminosity and darkened the skin colour *in vivo*, demonstrating to stimulate pigmentation intensity. In addition, the peptide also showed to be effective in prolonging the suntan two weeks after discontinuing the UV-exposure.

In conclusion, Melatime™ can be regarded as an effective tanning active ingredient for tanning accelerating and long-lasting cosmetic formulations, allowing to get a perfect long-lasting sun-kissed skin and shorten UV-exposure times.

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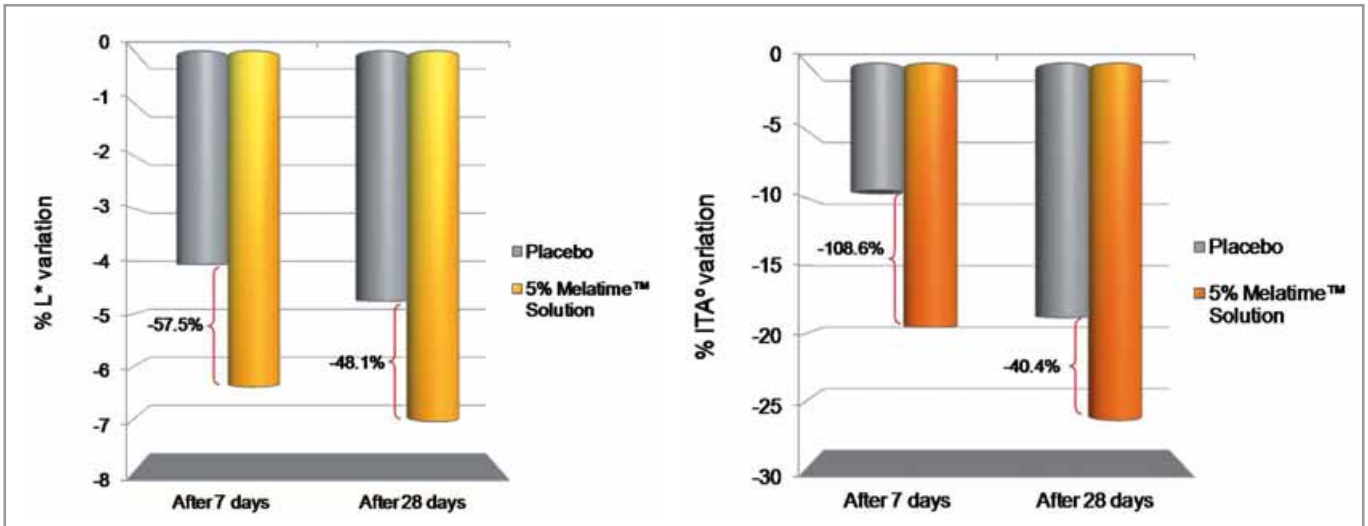

Fig. 6 Reduction percentages of L* and ITA° values after 7 and 28 days of treatment

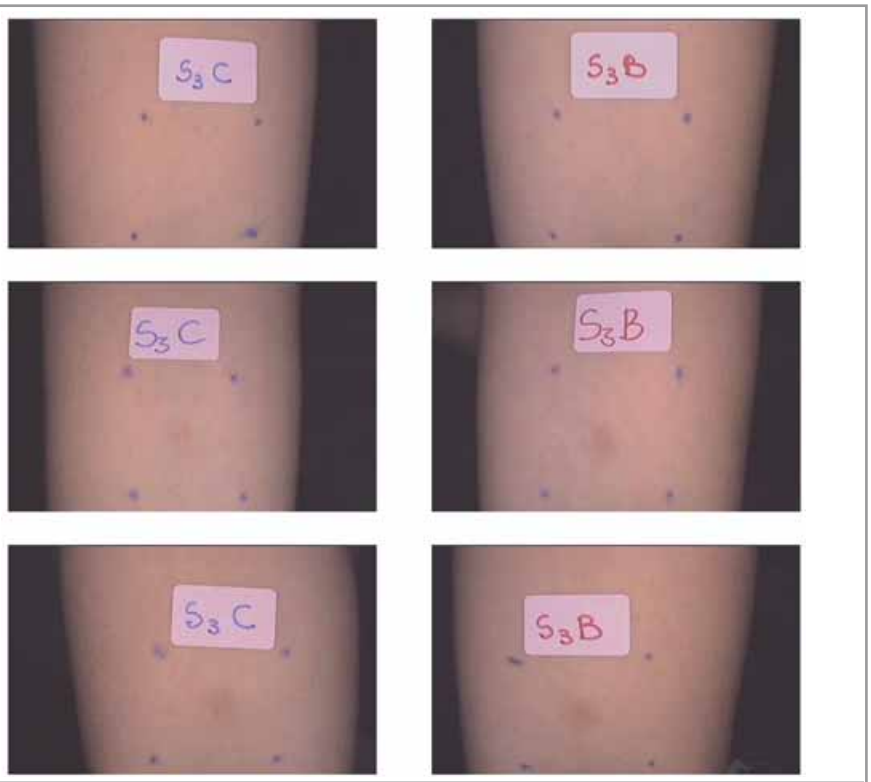

Fig. 7 Forearm images taken before the treatment, after 7 days of treatment and UV-irradiation and after 28 days of treatment (14 days from the last irradiation)

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Reconstructed Human Pigmented Epidermis (RHPE): An *in vitro* Model for the Evaluation of Melanogenesis

Introduction

In recent years, the reconstruction of various pigmented human skin culture systems is become more and more complicated. First reconstructed model was only composed by a two-dimensional culture system where normal or immortalized melanocytes were cultivated in combination with normal keratinocytes (5). Then the most advanced three-dimensional culture system was elaborated by co-seeding melanocytes and keratinocytes on an inert synthetic surface such as polycarbonate membrane (6) or on a dermal substrate composed of a dead de-epidermized dermis (7,8) or a collagen sponge colonized by fibroblasts (9). These pigmented human skin equivalents were cultivated at the air-liquid interface compared to the two-dimensional culture system reflecting more really physiological conditions of the human skin.

SkinEthic laboratories have developed an *in vitro* human model called Reconstructed Human Pigmented Epidermis (RHPE) consisting of a three-dimensional culture of normal melanocytes and keratinocytes in a chemically defined medium at the air-liquid interface.

RHPE model with its 3 different phototypes is a more relevant system to screen large numbers of putative melanogenic compounds and to subsequently evaluate their regulatory mechanisms on the melanogenesis.

RHPE Model Characteristics

When cultivated for 10-days on an inert 0.5 cm^2 polycarbonate filter at the air-liquid interface in chemically defined medium with appropriate melanocyte factors, normal human keratinocytes cultured in the presence of melanocytes of phototype II, IV and VI form the three-dimensional Reconstructed Human Pigmented Epidermis (RHPE) model. The different tanning degrees of these models correspond macroscopically to the three different phototypes of human skin **(Fig. 1)**. The different tanning degrees of this model correspond macroscopically

Abstract

The major source of skin color in humans is the melanin which plays an important role in photoprotection of human skin and in prevention of UV-induced photodamage. This pigment is produced in highly specialized cells, the melanocytes among the basal layer cells present in the epidermis and transferred to neighboring keratinocytes through melanosome maturation process (1). The pigmentary system is based on the close contact of melanocytes and keratinocytes forming the epidermal melanin unit (one melanocyte for approximately 36 keratinocytes).

The control of melanocyte homeostasis in human skin is regulated by the surrounding tissue environment. Several keratinocyte derived factors such as endothelin-1, basic fibroblast growth factor, stem cell factor or alpha melanocyte stimulating hormone are involved in regulating both the growth and the differentiation of melanocytes. Moreover, fibroblasts located in the dermal compartment, can also contribute to the modulation of melanocyte functions by the secretion of paracrine factors (2, 3, 4). Based upon the increased knowledge on these regulatory mechanisms, specific culture media were elaborated and melanocytes were amplified and maintained for a long term in culture. However, in order to assess modulators of pigmentation in vitro and to a large extent to better understand the pigmentary system regulation, keratinocytes/melanocytes co-culture systems should be preferred rather than pure melanocyte cultures.

to 3 different phototypes of human skin. RHPE model exhibits a homogeneous tanning demonstrating the functionality of the epidermal melanin unit *in vitro* and that the obtained phototype is determined by the melanocyte phototype, i.e. the rate of constitutive melanin synthesis.

The RHPE model presents a histological morphology comparable to the *in vivo* human tissue, consisting in the presence of a multi-layered, stratified and pigmented epidermis **(Fig. 2)**. Melanocytes are localized in the basal cell layer interspersed with basal cell keratinocytes. Moreover, melanin is distributed in the basal layer melanocytes and in upper layer keratinocytes similarly to that seen in normal human skin, reflecting its transfer into surrounding proliferating keratinocytes.

RHPE model is covered by a dense stratum corneum that allows the topical application of pigmentation modulators contrary to a two-dimensional culture system in which only systemic application is possible.

In summary, phototype VI RHPE model is recommended for studying the action of inhibitors of melanogenesis, whereas phototype II and IV RHPE models are suitable for evaluating the effect of stimulators of pigmentation.

■ Depigmentation Assay: Skin Care Cream Containing a Whitening Agent

1 µl of the formulation containing kojic acid are deposited daily onto the surface of the stratum corneum of the pigmented epidermal tissues of phototype VI (0.5 cm^2 surface) for 3 days. 48 hours after the last application (at day 5), tissues are investigated by histological techniques **(Fig. 3)**.

The repeated applications of cream containing kojic acid known as a good tyrosinase inhibitor give rise to a diminution of the tanning degree due to a decrease of melanin amount in the proportion of around 30 percent compared with untreated control. The rate of produced melanin can be also modulated by supplementing the culture medium with pigmentation inhibitors (data not shown).

■ Pigmentation Assay: UV Irradiation and/or Chemical Modulators

Human pigmented epidermal tissues of phototype IV are exposed daily to UVB irradiation at 50 mJ/cm^2, then to UVA irradiation at 1 J/cm^2 for 3 days (from day 12 to day 14 of culture) using a Vilber Lourmat RMX 3W irradiator, and cultivated in defined medium supplemented with pigmentation stimulators such as Melanotan I (MT-I) at different concentrations **(Fig. 4)**.

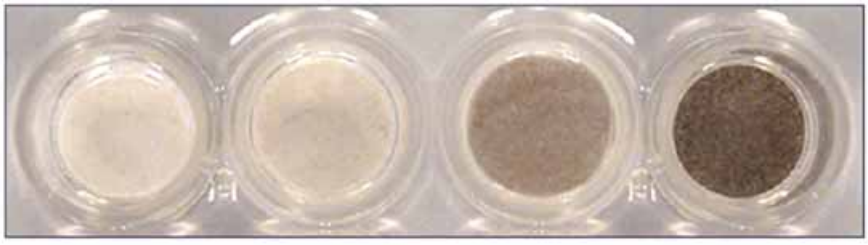

Fig. 1 Tanning degree of reconstructed human epidermis without melanocytes (a), and with melanocytes from phototypes II (b), IV (c) and VI (d)

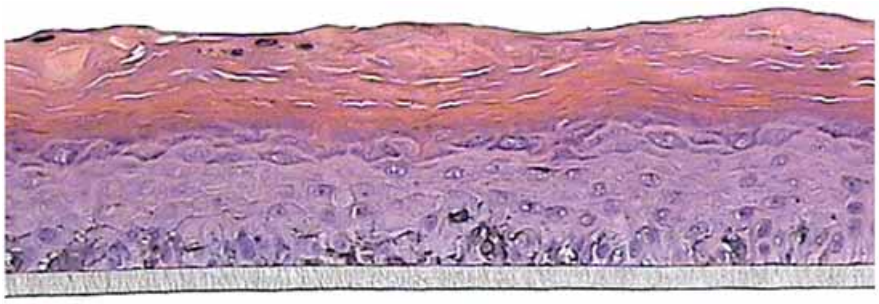

Fig. 2 Cross section of Reconstructed Human Pigmented Epidermis. Note the presence of melanocytes in the basal cell layer growing on the polycarbonate membrane

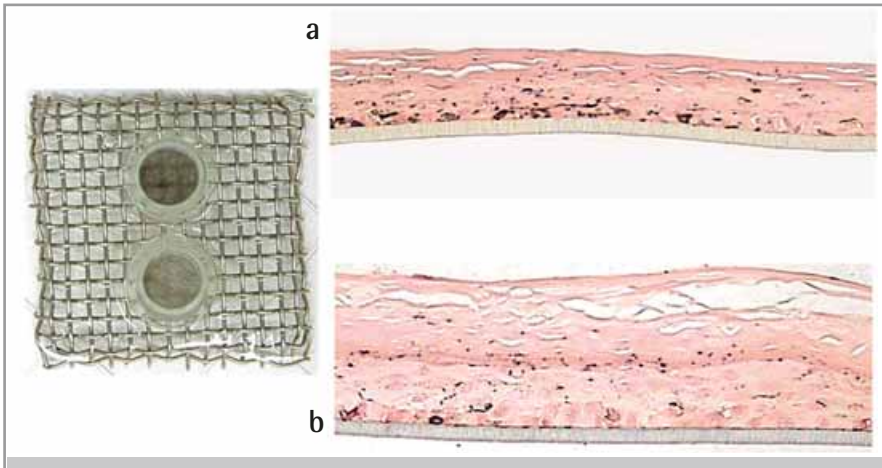

Fig. 3 Repeated applications of the skin care cream decrease the tanning degree (b) as compared with untreated control (a). Fontana Masson staining shows a correlation between reduced pigmentation and lower melanin content present in basal cell melanocytes and upper layer keratinocytes

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In this example, pigmentation is stimulated by UVA and UVB irradiation exposure and/or by the addition of a chemical compound, melanotan-I qualified as a potent pigmentation stimulator. In melanotan-I treated cases, the melanin synthesis increases in a dose-dependent way. It is also interesting to note that UV radiations potentiate melanotan-I treatment by triggering a cumulative effect on the induction of the melanin production.

Discussion

The RHPE model using a co-culture of normal human melanocytes and keratinocytes in a chemically defined medium that provides an improvement for screening numerous melanogenic compounds. The presence of a stratum corneum allows the topical application of test molecules onto the surface of the epidermal pigmented construct and permits a better evaluation of their efficacy for modulating the melanogenesis.
RHPE model with its 3 different phototypes should exhibit a great potential in the cosmetic field not only for discovering whitening agents used for the treatment of irregular hyperpigmentation such as melasma, freckles and age spots, but also for assessing new molecules playing a role in protection against sun exposure such as sunscreens and self tanners.
Recently, it has been recognized that the process of skin reconstruction presents an innovative technology in several domains and gives promising insights onto the study of acquired disorders and pigmentary diseases. Thus, comparing reconstructs have been made with vitiligo versus normal melanocytes and using the same normal keratinocytes in order to study the possible causative disparition of melanocytes in vitiligo (10). Moreover, pigmented reconstructs have been also realized for understanding regulatory mechanisms involved in melanoma by using genetically modified melanocytes (11) and also for elaborating clinical models by knocking down specific genes after the transfection of melanocytes with their equivalent siRNAs (12).
SkinEthic also commercializes other reconstructed human tissues such as epidermis (RHE and EpiSkin), and mucosa (corneal, gingival, oral and vaginal). These 3D reconstructed human tissues are based on a 20-year cumulated experience on tissue engineering allowing us to serve our customers worldwide with high quality screening tools for efficacy and safety screening.

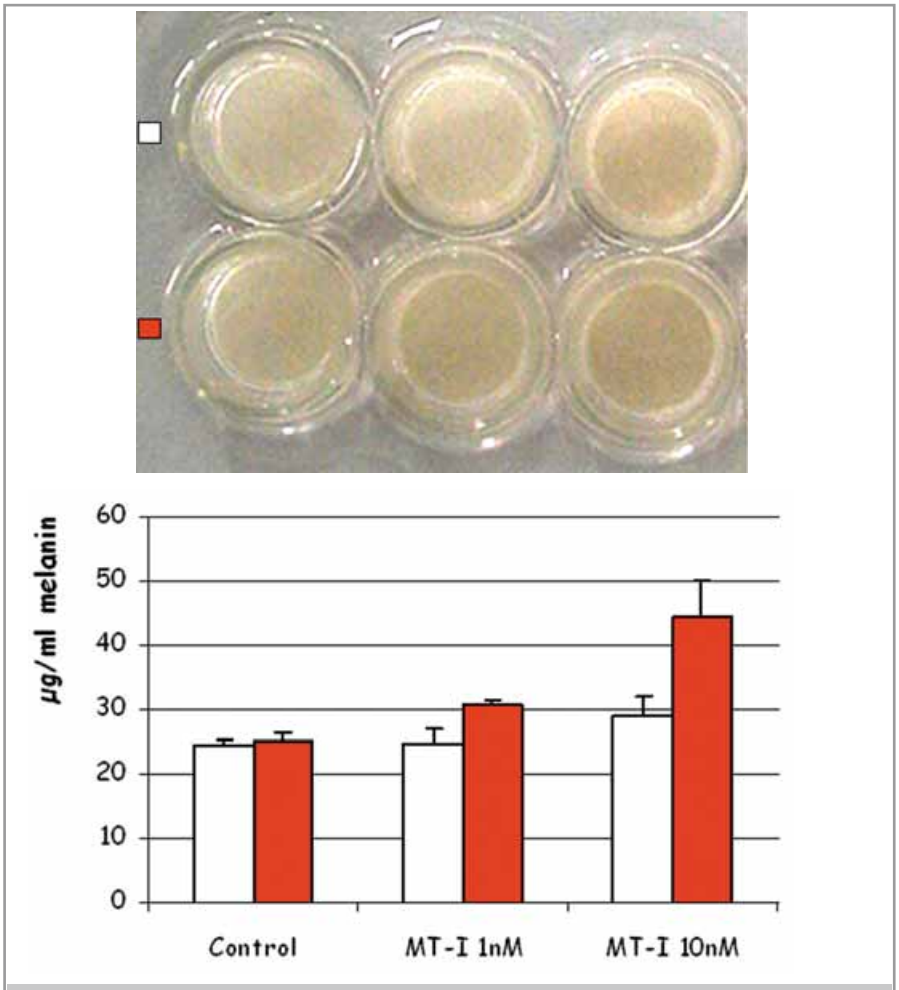

Fig. 4 The pigmentation is induced by UVA and UVB irradiation (red square) compared with untreated control (white square). Addition of MT-I leads also to an increase of pigmentation. These observations are confirmed by colorimetric quantification performed after melanin extraction

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A Well-Formulated Sunscreen for the Right Protection

Introduction

The solar radiation that reaches the earth's surface is composed of ultraviolet radiation (UV), visible light (VIS) and infrared radiation (IR) **(Table 1)**. In addition to the desired effects of sunlight on vitamin D3 synthesis and pigmentation of the skin, excessive and / or unprotected stay in the sun can have adverse effects. In particular, the comparatively low proportion of Ultraviolet radiation is a threat to human skin and the whole organism. Which effects UV rays have on or in the skin, depends on their wavelength. UV-B radiation with wavelengths below 300 nm can penetrate the entire epidermis. The longer-waved UV-A radiation can penetrate to the middle layers of the connective tissue (dermis) (1).
The UV-C radiation is not present in the terrestrial solar spectrum but would not cause any skin damage due to its low penetration rate. In order to protect itself from excessive exposure to light, the human skin has various adaptation mechanisms. Besides the formation of a so-called light callus (radiation-related thickening of the horny layer), the skin forms the pigment melanin either due to indirect (UV-B) or direct (UV-A) pigmentation of the skin. Through the absorption and scattering of UV radiation by the macromolecule, the nuclei and thus the genetic code of the cells are protected from harmful changes (2). Moreover, due to biochemical adaptation by immediate consumption of existing mediators in the skin the light tolerance can be increased. In addition, repair mechanisms to correct any subsequent DNA damage are activated. The protection mechanisms in the body can however be overloaded in the event of intense UV exposure. Cosmetic and dermatological light protection agents, therefore, have the task to relief this body's own defence mechanisms and to prevent the development of acute and chronic light damages.
The acute effects of an overdose of UV-B radiation is the formation of erythema (3). UV-B-related damage in the skin is mainly caused by the release of mediators (histamine, kinin, prostaglandins), a degradation of the DNA structures in the cell nuclei and the formation of free reactive radicals (2).
UV-A radiation has a significant influence on the occurrence of premature skin aging (4) and skin cancer (5). More-

	Wavelength range [nm]
Ultraviolett	200-400
UV-C	200-285
UV-B	285-320
UV-A	320-400
Visible Light	400-800
Infrared	800-3000

Table 1 Spectrum of sunlight on the earth

Summary

Because of the pathological effects of UV radiation on the skin there is a great need for highly-protective and also with regard to contact allergies and light dermatosis safe sunscreens. Consumers want to be sure that they are well protected while being in the sun for sun bathing or during swimming or other sporting activities. UV filters (UV-absorbent or reflective substances) protect the skin against damage such as sunburn, premature aging, skin cancer and UV light-induced sensitivity. Various organic UV absorbers and insoluble, inorganic filters are used for this purpose. In addition to the actual UV filters, the cosmetic formulation, the matrix, is crucial. This article describes the influence of different emulsifiers, polymers and various stabilizing additives on the SPF of a cosmetic formulation are described.

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over, UV-A rays can cause phenomena such as the polymorphic light dermatosis (6, 7) due to oxidative stress, which overloads the natural antioxidant system at unphysiological high doses. Under the term »polymorphic light dermatosis« a whole range of illnesses are subsumed. Their common cause is mainly seen in immunomodulatory effects caused by formation of reactive oxygen species (ROS) induced by UV-A radiation (8). Another form of pathological light reactions is the erythematous light dermatosis (Mallorca acne). Unlike polymorphic light dermatosis the cause of the Mallorca acne is in a combined exposure of UV-A radiation and unsaturated lipids or specific emulsifiers and formation of peroxides, which irritate the follicles. The occurrence of Mallorca acne can be prevented by the use of suitable sun protection, which are formulated without unsaturated lipids and emulsifiers (9).
Due to the pathological effects of UV radiation on the skin there is a great need for highly protective and also safe sunscreens with regard to contact allergies (10) and light dermatosis. UV-absorbing substances protect against skin damage such as sunburn, premature aging (11), skin (12) and UV-induced contact hypersensitivity (13). Various organic UV absorbers are used for this purpose, primarily soluble organic UV-A, UV-B and broadband filters, as well as insoluble, inorganic filters (14). These so-called physical light filter (TiO_2, ZnO) are used more and more in recent times due to their good performance in sun protection and their excellent skin compatibility (15-18).
As a feature for the UV-B protection of sun protection formulations serves the sun protection factor (SPF), the ratio of the dose needed for erythrem formation on protected to unprotected skin (19).
The regulation and declaration of sunscreen products, as well as methods to test their effectiveness is varies in different regions / countries. In 2006 Japan, South Africa and Europe agreed on the international test method according to Colipa 2006 to determine the sun protection factor *in vivo*. The FDA describes the U.S. test method in the monograph »Sunscreen Drugs« (Federal Register Vol 64 No. 98 May 21, 1999 pages 27666-693). Australian test methods are approved by the TGA (Therapeutic Goods Administration). The currently used *in vivo* SPF method in Australia dates from 1998.
The amount of UV-A protection should be adjusted to the particular sun protection factor of the formulation to ensure optimal protection of the skin against UV-A radiation. In Europe, the UVA protection needs to be at least 1 / 3 of the SPF claimed and the critical wavelength needs to be greater than 370 nm. In order to determine the UVA protection performance, the PPD method (Persistent Pigment Darkening) or a comparable in vitro method is used in Europe. In the U.S. the UVA test method is still under development at FDA. Japan uses a test method for determining the UVA protection similar to Europe. Australia uses an *in vitro* test method.
The article by *David C. Steinberg* (20) describes the differences in test methods.

The Perfect Sun Cream

The perfect sun lotion protects sufficiently and long-lasting against UVA and UVB rays, is water resistant, easy to use, quickly absorbed, good smelling, has a pleasant feeling on the skin, does not show whitening, is not sticky after absorption, easily distributable, stable, non-sensitizing and otherwise toxicologically safe and well preserved.
To combine all of these properties in one formulation in order to meet consumer satisfaction is a challenge for all sunscreen manufacturers.
In the development of sunscreen formulations difficulties, such as crystallization of organic filters, or emulsifiers, bad sensorics, physical instability (phase separation), not enough SPF or UVA protection, occur and are a challenge for the formulator; the challenge is even more the higher the SPF of sun cream should be. Given this challenge, not only the choice of sun filters and their combination is important; it is necessary to investigate the various influences of the other ingredients in the sunscreen formulation on the SPF, as well as the stability and the way of combination of individual ingredients. The following studies are an introduction to investigate the skeleton of a sunscreen formulation to assess the effect of various polymers and silicones on the SPF of a cosmetic formulation.

Materials and Methods

Test formulation 1:
Composition
(organic and inorganic UV-filters):

C12-15 Alkyl Benzoate	8 %
Caprylic/ Capric Triglyceride	5 %
Octocrylene	9 %
Ethylhexyl Methoxycinamate	7 %
Butyl Methoxydibenzoylmethane	2.5 %
Titanum Dioxide	5 %
Potassium Cetyl Phosphate	3 %
Cetearyl Alcohol	1 %
Sunflower Seed Sorbitol Esters	2 %
Polymer	0.6 %
Water	ad 100 %

The SPF of the formulation was calculated using the Ciba Sunscreen simulator to SPF 30 (21).
For the determination of the SPF the international SPF test method according to Colipa 2006 (*in vivo*) was used.
In the above **test formulation 1**, the following polymers and polymer combinations have been investigated:

Aristoflex® AVC
(INCI: Ammonium Acryloyldimethyltaurate / VP Copolymer

Aristoflex® AVS
(INCI: Sodium Acryloyldimethyltaurate / VP Copolymer)

Aristoflex® HMB
(INCI: Ammonium Acryloyldimethyltaurate / Beheneth-25 Methacrylate Crosspolymer)

Aristoflex® BLV
(INCI: Ammonium Acryloyldimethyltaurate / Beheneth-25 Methacrylate Crosspolymer)

Carbopol® 980
(INCI: Carbomer)

Carbopol® ETD 2020
(INCI: Acrylates / C 10-30-Alkyl Acrylates-Crosspolymer)

Pemulen® TR 2
(INCI: Acrylates / C 10-30-Alkyl Acrylates-Crosspolymer)

Xanthan Gum
Aristoflex® AVC 1:1 [g/g] combined with
- Xanthan Gum
- Genopur® KW 3500 D (polymerer Phosphorsäureester)
- Genapol® DAT 100 (PEG-150 Polyglyceryl-2 Tristearate)

Aristoflex® BLV 1:1 [g/g] combined with
- Aristoflex® AVS
- Aristoflex® HMB
- Aristoflex® AVC
- Carbopol® 980

Pemulen® TR 2 1:1 [g/g] combined with
- Aristoflex® HMB
- Carbopol® 980
- Carbopol® ETD 2020

Test formulation 2:
Composition
(without physical UV-filters):

C12-15 Alkyl Benzoate	8 %
Caprylic/Capric Triglyceride	5 %
Caprylyl Methicone	3 %
Octocrylene	9 %
Ethylhexyl Methoxycinnamate	7 %
Butyl Methoxydibenzoylmethane	2.5 %
Emulgator	1.0 %
Polymer variable	
Water	ad 100 %

The SPF of the formulation was calculated with the Ciba Sunscreen simulator to SPF 20 (21).
For the determination of the SPF the international SPF test method according to Colipa 2006 (*in vivo*) was used.
In the above **test formulation 2** the following silicones have been tested:

Caprylyl Methicone (SilCare® Silicone 41M15)	3 %
Trideceth- 9 PG- Amodimethicone (and) Trideceth- 12 (SilCare® Silicone SEA)	1.5 %

Discussion of results on test formulation 1
Fig. 1 shows that Aristoflex® AVC achieved the highest SPF of 53 in the test formulation.
Pemulen® TR 2 achieved an SPF of 50, Aristoflex® AVS an SPF 42.
Carbopol® ETD 2020 and xanthan gum obtained the calculated SPF of 30.
Carbopol® 980 has remained below the calculated SPF at 24.
In **Fig. 2** Aristoflex® AVC is compared to polymer mixtures of Aristoflex® AVC with other polymers.
One can see that the addition of xanthan gum, Genopur® KW 3500 D (polymeric phosphoric acid ester) or Genapol® DAT 100 (PEG-150 Polyglyceryl-2 tristearate) decreases the SPF to the pure Aristoflex® AVC formulation.
Fig. 3 shows the comparison of Aristoflex® BLV, a polymeric emulsifier, to Aristoflex® BLV combinations. The addition of Aristoflex® AVS and Aristoflex® HMB increase the SPF in comparison to Aristoflex® BLV alone. The addition of Aristoflex® AVC and Carbopol® 980 decreases the SPF in comparison to Aristoflex® BLV alone.
The combination of Aristoflex® BLV and Aristoflex® HMB results in a SPF of 52, which is the second-highest value in the test series, whereas the combination of Aristoflex® HMB with Pemulen® TR2 achieves the calculated SPF of 30 only.
In **Fig. 4** the comparison of the polymeric emulsifier Pemulen® TR 2 against a combination of Pemulen® TR 2 with polymeric thickeners is presented. The combinations of Pemulen® TR 2 with Aristoflex® HMB, Carbopol® ETD 2020 and Carbopol® 980 decrease the SPF compared to Pemulen® TR 2 alone.
Fig. 5 shows the combination of a polymeric thickener Aristoflex® HMB with the polymeric emulsifiers Pemulen® TR 2 and Aristoflex® BLV. The combination with Aristoflex® BLV results in a significantly higher SPF of 52, whereas the

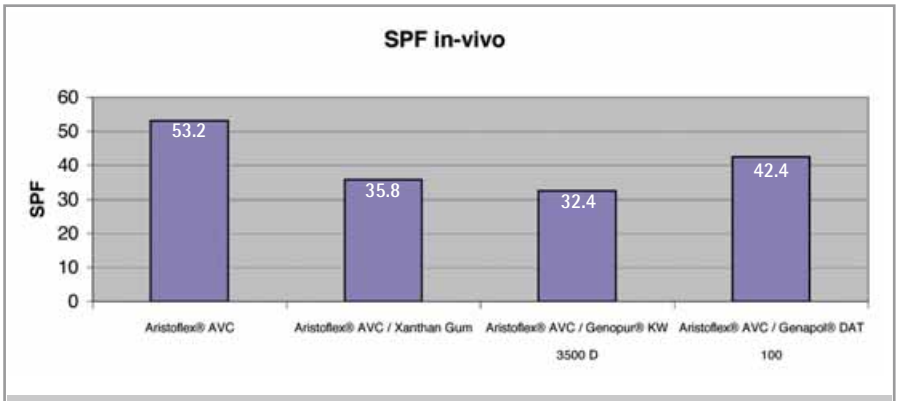

Fig. 1 SPF *in vivo* of test formulation 1

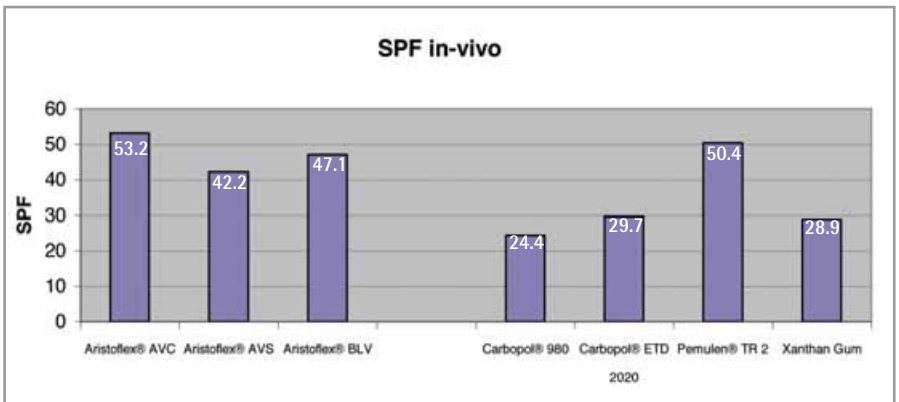

Fig. 2 SPF *in vivo* of test formulation 1

combination with Pemulen® TR 2 reaches the calculated SPF of 30 only.
The test formulations with different polymers and polymer combinations result in different emulsion types **(Table 2)**. The type of emulsion does not directly correlate with the achieved SPF. The results show that gelling agents for the water phase of an emulsion stabilize not only O/W emulsions, but can also be used in W/O emulsions in order to stabilize the formulation.
Patent application WO 02 / 051377 describes that W/O emulsions stabilized with Ammonium Acryloyldimethylaturate / Vinylpyrrolidone Copolymers show the following advantages:

- better moisturizing effect
- easier to formulate
- promotion of better skin smoothness
- better skin care effect
- serve as a vehicle for cosmetic and medical dermatology actives
- better sensory properties, such as the spreadability and absorption on the skin
- higher stability against phase separation into oil and water phase
- improved biocompatibility

The skin feel of emulsions with different polymers have been evaluated in a panel test
Aristoflex® AVC
Aristoflex® HMB
Carbopol® 980
Carbopol® ETD 2020
Xanthan Gum

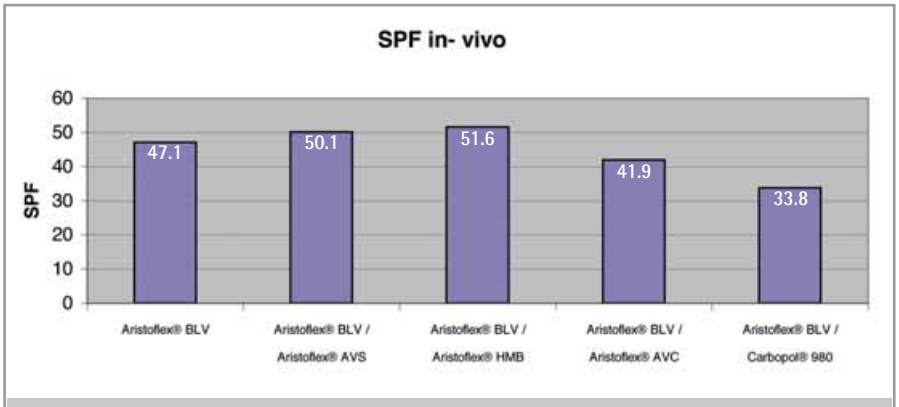

Fig. 3 SPF *in vivo* of test formulation 1

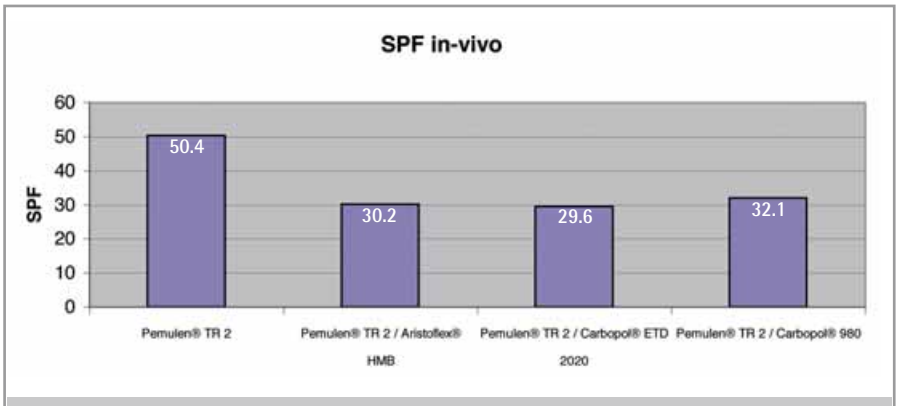

Fig. 4 SPF *in vivo* of test formulation 1

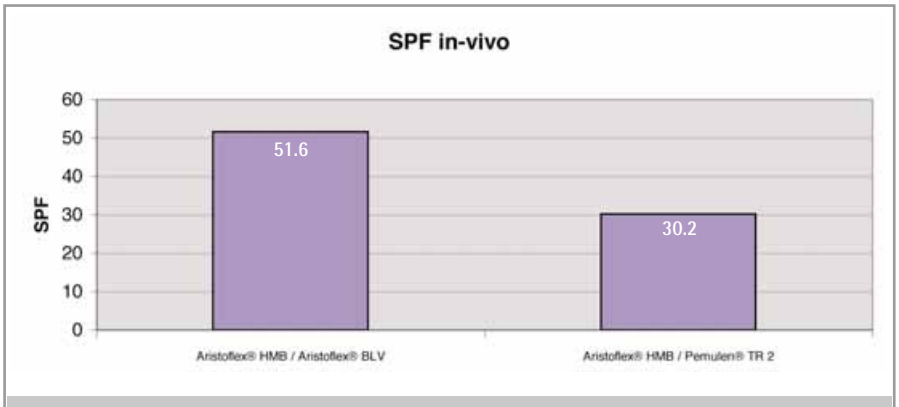

Fig. 5 SPF *in vivo* of test formulation 1

Aristoflex®AVC and HMB have been assessed to provide the best skin feel from the male test persons. Further Aristoflex® AVC has been described as non-sticky and not oily. The spreadability of the sunscreen formulation was best with Aristoflex®HMB. Gloss and appearance of the **sunscreen formulations 1** and **2** were judged the best with Carbopol® 980 and Aristoflex® HMB by the male test persons. Aristoflex® AVS showed the best skin smoothness **(Table 3** and **Fig. 6)**.
The female test persons evaluated the appearance and gloss of the formulation with Carbopol® 980 best. The Acryloyldimethyltaurat copolymers were better assessed in terms of stickiness, and showed a less greasy feeling on the skin, the overall skin feel was assessed best with the Carbopol® 980 by the female test persons **(Table 4** and **Fig. 7)**.
Furthermore, in the test series, the polymer Aristoflex® BLV has been found to be particularly suitable for sprayable sunscreen formulations. It provides stability at relatively low viscosity to the spray formulation **(Formulation 2)**.

Sample No	Polymer or Polymer Combinations	Mean SPF	Emulsion typ
6	Aristoflex® AVC	53.2	W/O
7	Carbopol® 980	24.4	W/O
8	Carbopol® ETD 2020	29.7	W/O
9	Pemulen® TR 2	50.4	W/O
10	Aristoflex® AVS	42.2	W/O
11	Aristoflex® BLV	47.1	W/O
12	Xanthan Gum	28.9	O/W and W/O Mixture
13	Aristoflex® AVS/ BLV	50.1	W/O
14	Aristflex® HMB/ BLV	51.6	W/O
15	Aristoflex® AVC/ Xanthan Gum	35.8	O/W and W/O Mixture
16	Aristoflex® AVC/ Genapopur® KW 3500 D	32.4	O/W and W/O Mixture
17	Aristoflex® AVC/ Genapol® DAT 100	42.4	O/W
18	Aristoflex® HMB/ Pemulen® TR 2	30.2	W/O
19	Carbopol® ETD 2020/ Pemulen® TR 2	29.6	W/O
20	Carbopol® 980/ Pemulen® TR 2	32.1	W/O
21	Carbopol® 980/ Aristoflex® BLV	33.8	W/O
22	Aristoflex® AVC/ BLV	41.9	W/O

Table 2 SPF *in vivo* and emulsion type of test formulation 1

Phase	Ingredient	INCI name	%
A	Tegosoft TN	C12-15 Alkyl Benzoate	8.00
	Velsan® CCT [1)]	Caprylic/Capric Triglyceride	5.00
	Eusolex OCR	Octocrylene	9.00
	Eusolex 2292	Ethylhexyl Methoxycinnamate	7.00
	Eusolex 9020	Butyl Methoxydibenzoylmethane	2.50
	Cetearyl Alcohol		1.00
	Hostacerin® SFO[1)]	Sunflower Seed Oil Sorbitol Esters	2.00
	Phenonip®[1)]	Phenoxyethanol (and) Methylparaben (and) Ethylparaben (and) Butylparaben (and) Propylparaben (and) Isobutylparaben	q.s.
	UV Titan M 262	Titanium Dioxide (and) Alumina (and) Dimethicone	5.00
	Hostaphat® CK 100[1)]	Potassium Cetyl Phosphate	3.00
B	Arisoflex® AVC[1)]	Ammonium Acryloyldimethyltaurate/VP Copolmer	0.60
C	Water	Aqua	ad 100.00

[1)] Clariant

Procedure:

I. Melt the components of A at approx. 70°C.
II. Heating C to approx. 70°C.
III. Add B to I and stir for 2 minutes with 30 rpm.
IV. Finally add II to III, stir with approx. 600 rpm until cool.

Results:

pH: 5,30
Viscosity (Brookfield, 20°C 20 rpm): 25100 mPas
Appearance: soft white cream
Stability: 3 months at RT, 40°C and 0°C.

Guide Recipe 1 W/O Sunscreen Cream. SPF 50 (*in vivo* according to Colipa 2006) non-sticky, non-greasy

Phase	Ingredient	INCI name	%
A	Hostaphat® KL 340 D [1)]	Trilaureth-4 Phosphate	2.00
	Octyldodecanol		4.50
	Eusolex® 2292	Ethylhexyl Methoxycinnamate	10.00
	Eusolex® 9020	Butyl Methoxydibenzoylmethane	5.00
	Eusolex® 6300	4-Methylbenzylidene Champhor	4.00
	SilCare® Silicone 41M15 [1)]	Caprylyl Methicone	2.00
B	Arisoflex® BLV [1)]	Ammonium Acryloyldimethyltaurate/Beheneth-25 Methacrylate Crosspolymer	0.20
C	Water	Aqua	40.00
D	Water	Aqua	ad 100%
	Glycerin		3.00
	Panthenol		0.50
E	Alcohol		5.00
	Tocopheryl Acetate		0.50
	Fragrance		q.s.
	Nipa® Preservative [1)]		q.s.

[1)] Clariant

Procedure:

I. Melt A at approx. 80°C
II. Then add B to I.
III. Heat C to approx. 80°C
IV. Stir III into I with high speed dispersion tool (Ultraturrax/staro) for about 2 min.
V. Stir with an Anchorstirrer (200 rpm) until cool (35°C) and add the components of D.
VI. Stir E into V and stir 1 hour.
VII. If necessary adjust the pH.
VIII Finally homogenize the emulsion.

Results:

pH: 6,70
Viscosity (Brookfield, 20°C 20 rpm): 600 mPas
Appearance: low viscosity white emulsion
Stability: 3 months at RT, 40°C, 45°C and 50°C.

Guide Recipt 2 O/W-Sun Sprayable Emulsion. Calculated SPF 17, low viscosity sprayable sun screen

Male Probands	Carbopol® 980	Carbopol® ETD 2020	Xanthan Gum	Aristoflex® AVC	Aristoflex® HMB	Aristoflex® AVS
Appearance	4	0	3	3	4	1
Shine	4	1	2	3	4	1
Dosage	3	4	4	4	3	3
Spreadability	3	2	3	3	4	3
Absorption	2	1	1	3	3	3
Stickness	2	4	2	4	3	3
Smoothness	3	3	2	3	3	4
Oily/Fatty Feeling	2	4	2	4	3	3
Moisturizing	3	4	3	3	3	3
Skin Feeling Sumary	2	3	2	4	4	3

Table 3 Sensory assessment of test formulation 1 by male panelists (Skale 0 to 4, 0 worst evaluation, 4 best evaluation by the test panel)

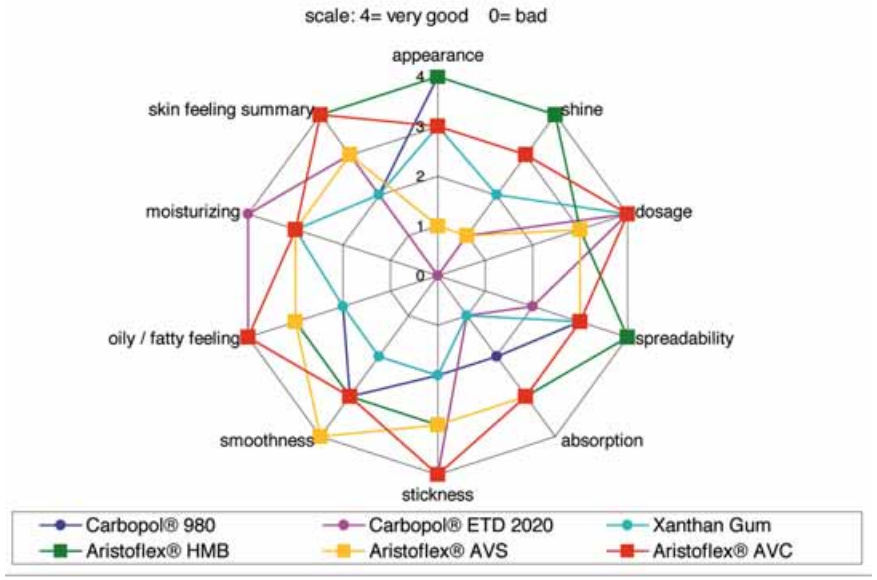

Fig. 7 Organoleptic testing of sunscreen formulations (male probands)

Discussion of results to test formulation 2

Fig. 8 shows, that SilCare® Silicone 41M15 at a use concentration of 3 % increased the SPF of the formulation in comparison to the formulation without silicone. SilCare® Silicone SEA increased the SPF more than 41M15, although it was used at half of the concentration, at 1.5% only.

Conclusion

In summary, one can say that the development of the perfect sunscreen formulation is still a challenge for every developer, since only the right combination of all ingredients (thickeners, stabilizers, emulsifiers, oils, UV filters, etc.) in the right concentration results in a perfect formulation.

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Female Probands	Carbopol® 980	Carbopol® ETD 2020	Xanthan Gum	Aristoflex® AVC	Aristoflex® HMB	Aristoflex® AVS
Appearance	4	0	2	3	3	2
Shine	4	2	1	2	3	1
Dosage	4	4	4	4	4	4
Spreadability	3	3	3	3	3	3
Absorption	2	2	3	3	2	3
Stickness	2	4	2	3	3	3
Smoothness	2	3	2	3	3	2
Oily/Fatty Feeling	2	3	3	3	3	3
Moisturizing	4	3	2	3	3	3
Skin Feeling Summary	3	3	2	3	3	3

Table 4 Sensory assessment of test formulation 1 by female panelists (Skale 0 to 4, 0 worst evaluation, 4 best evaluation by the test panel)

The Acryloyldimethyltaurate copolymers used in the study are novel, synthetic polymers, which in recent years have gained an important role as gelling agents for aqueous systems and as stabilizers and texturizers for O/W emulsions. Besides natural thickeners such as cellulose-derivatives or xanthan gum and besides synthetic thickeners such as the classical polyacrylates – the Acryloyldimethyltaurate copolymers have developed to a market standard in the cosmetic industry. They show an excellent property profile, are useable in a broad pH range, stable against shear stress and UV irradiation, have excellent tactile properties, serve as the basis for modern formulation concepts and can in addition to the known properties provide outstanding results in terms of the SPF in sunscreen formulations. The present study shows, that these rheology modifiers also increase stability and SPF in W/O sunscreens.

The emulsifier combination of Sunflower Seed Oil Sorbitol Esters and Potassium Cetyl Phosphate provides a stable base for the exchange of the polymers in **test formulation 1**. Potassium Cetyl Phosphate is widely used in sunscreen formulations to enhance the water resistance of the formulation.

From the experiments with test formulation 1 the Guide Recipe 1 was obtained with SPF 50 (Colipa 2006 *in vivo*). This

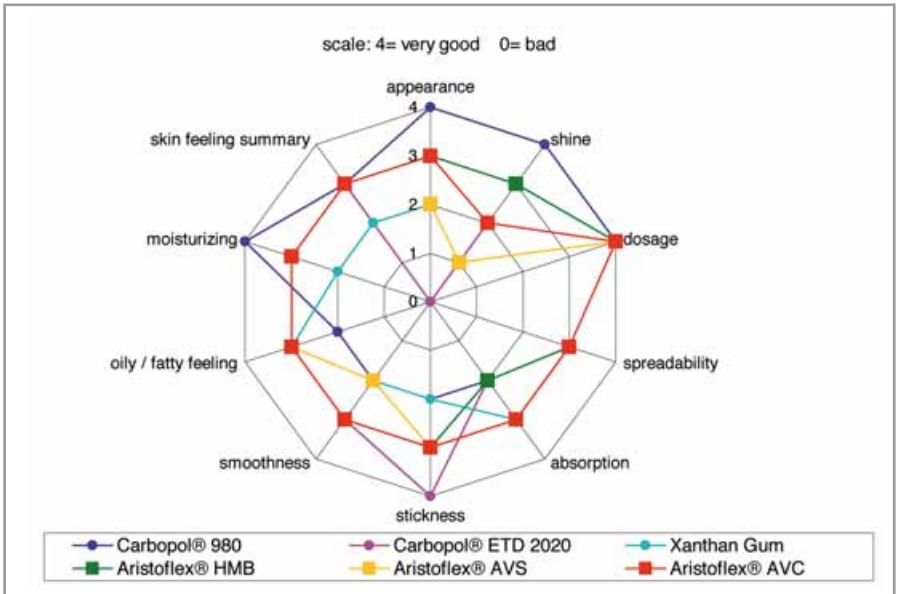

Fig. 8 Organoleptic testing of sunscreen formulations (female probands)

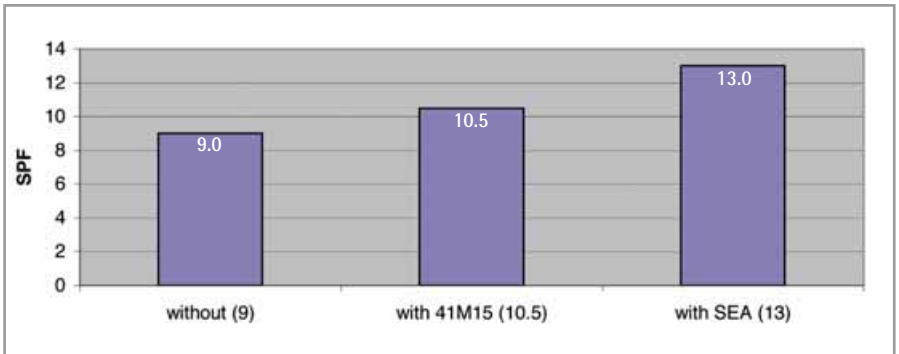

Fig. 8 Influence of SilCare® Silicone 41M15 and SEA

formulation is optimized in its composition, so it shows very good skin feeling, no stickiness and has a high sun protection.

SilCare® Silicones (Caprylyl Methicone or Trideceth-9 PG-Amodimethicone) do not only improve the skin feeling of a cosmetic emulsion, but also increase the SPF of a formulation.

With Aristoflex® polymers and SilCare® silicones, and the Clariant emulsifiers/co-emulsifiers high quality sunscreen formulations with outstanding skin feel and very good stability are possible, which in addition meet the requirements for high sun protection.

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New Applications for the Aboriginal Remedy: Tea Tree Oil – Wrapped up in Sugar Molecules

Tee Tree Oil as a General-Purpose Household Remedy

For the last ten years or so, natural cosmetic preparations have proved a big hit with consumers – reason enough for product developers to be constantly looking for new plant-based actives. Now, their attention has turned to tea tree oil – an essential oil known for its powerful action against a broad spectrum of bacteria and other microorganisms. Many use tea tree oil as a general-purpose household remedy. Although not permitted as a pharmaceutical in Germany, Austria and Switzerland, tea tree oil can be used in cosmetics.

All the same, tea tree oil has certain drawbacks that prevent its use in cosmetics. For one thing, it is highly volatile. As soon as the container is opened, a considerable amount of the tea tree oil evaporates and the benefit is lost. Many are also put off by the oil's pungent smell. Moreover, tea tree oil is sensitive to oxidation. This process produces compounds that can trigger local skin irritations and contact allergies. For these reasons, product developers have been wary of using tea tree oil. Many of the potential applications for this essential oil proved impracticable – until WACKER succeeded in solving the problem.

Tea tree oil is extracted from the leaves and branch tips of the Australian tea tree of the genus *Melaleuca alternifolia* by steam distillation. The colorless to pale yellowish-green oil has a characteristic, spicy smell redolent of eucalyptus, camphor and nutmeg. Tea tree oil evaporates quickly, i.e. it is highly volatile. This type of essential oil, unlike fatty oils such as sunflower oil, does not leave greasy stains on paper or textiles.

Like all essential oils, tea tree oil is a complex mixture of substances. It comprises over 100 compounds, about 60 of which have been identified so far. Tea tree oil's chief constituents include the monoterpenes terpinen-4-ol, γ-terpinene and α-terpinene. The oil's composition is influenced by many factors, such as the variety of tea tree, its location and prevalent climatic conditions, the age of the harvested leaves, and the time of harvest.

Introduction

Australian tea tree oil is an extremely effective antiseptic. However, the Aboriginal remedy has its disadvantages: It evaporates quickly, has a strong smell and is chemically sensitive. In the past, these properties posed an obstacle to the oil's use in cosmetic formulations, personal care products and coatings. Now, the new WACKER technology of microencapsulation in cyclodextrin has opened up a broad range of uses for this valuable oil.

Even the distillation procedure affects the oil composition. Tea tree oils with a high terpinen-4-ol content are considered especially effective, biologically speaking.

Tea Tree Oil - The Aboriginal Remedy

What we know about tea tree oil's antimicrobial effects ulti-mately dates back to the Australian Aborigines. They traditionally live in the northern part of present-day New South Wales – the tea tree's natural habitat – and have been using tea tree leaves for medicinal purposes since ancient times. The oil's antiseptic effect was first investigated by scientists in the 1920s. They found that tea tree oil's disinfecting action was about eleven times as strong as that of phenol, which was the most powerful disinfectant in common use at that time. In the 1940s, this traditional remedy lost ground to modern antibiotics such as penicillin. When it was shown in the 1970s that bacteria could develop resistance to modern antibiotics, the time was ripe for tea tree oil's renaissance.

Lab Studies Confirm the Antimicrobial Effects

Recent lab studies and clinical trials confirm the antimicrobial activity of terpinen-4-ol-rich tea tree oil. They showed that tea tree oil is also effective against bacterial strains resistant to anti-biotics. Additionally, it is unlikely that bacteria will build up resistance to tea tree oil.

Our natural skin bacteria are less sensitive to tea tree oil than pathogenic organisms.

The Problem: The Oil Needs Protecting

What sets tea tree oil apart is its truly broad spectrum of action: it exhibits antiviral activity and acts as a bactericide, fungi-cide and insect repellent. When applied topically, preparations containing tea tree oil counteract, for example, acne, dandruff, athlete's foot, fungal nail infections, yeast infections and cold sores; in low concentrations, the oil is suitable for preserving cosmetic and personal-care preparations. Tea tree oil repels flies, mosquitoes, head and body lice, ticks and itch mites; in higher concentrations, it even kills these harmful arthropods.
Tea tree oil contains chemically-sensitive substances. Several oil constituents oxidize on contact with air at room temperature, especially when there's light, too. In this way, air greatly reduces the terpinen-4-ol content (main active). What's worse is that the gradual loss of active constituents is accompanied by a dramatic rise in the concentration of substances, such as p-cymol, ascaridol and 1,2,4-trihydroxymenthane, which irritate the skin and are conducive to allergic reactions.
To minimize the risk of skin irritation and allergic contact eczema, the German Federal Institute of Risk Assessment (BfR) recommends a maximum tea tree oil concentration in cosmetics of 1 wt%. »*In many instances, such a low tea tree oil content makes it impossible to reliably obtain the desired effect*«, says *Marlies Regiert*, responsible for cyclodextrin-product development at WACKER FINE CHEMICALS.

The Solution: Inclusion in Cyclodextrin

One way out of this predicament is the molecular inclusion of tea tree oil in a suitable cyclodextrin – a method that has proved effective for fragrances, vitamins A, E and F as well as other lipophilic substances. Cyclodextrins are ring-shaped sugar molecules comprising several interlinked glucose units. Each cyclodextrin molecule can house a lipophilic guest molecule in its cavity, and will release it again under suitable conditions. Regiert explains the principle: »*It's best to imagine a cyclodextrin molecule as a tiny beauty case in which an individual molecule is kept safe and protected against the influence of oxygen, light and heat. When necessary, the case is opened and the molecule emerges completely unchanged – as fresh as when it was put in the case. The key to opening these molecular cases is moisture*«.
For as long as the tea tree oil remains enclosed in the cyclodextrin, it enjoys perfect protection – it can neither evaporate nor be altered chemically. »*The skin's natural moisture and temperature are sufficient to release the tea tree oil. The oil thus reaches the skin in juvenile form. There are no skin-irritating and sensitizing oxidation and degradation products*«, she adds.
Regiert conducted a battery of experiments to ascertain the extent to which the molecules of the tea tree oil constituents are protected within the cyclodextrin complexes. She not only tested the inclusion compounds themselves, but also examined the behavior of the complexes in cosmetic formulations and surface coatings, such as emulsion paints with repellent action.
β-Cyclodextrin, which comprises seven glucose units, plays a key role in affording protection against air, UV light and

Fig. 1 Tea tree oil, an effective natural antimicrobial agent with a broad spectrum of action, is extracted from the Australian tea tree (*Photo*: Wacker Chemie AG)

Fig. 2 Taking an olfactory sample from a β-cylodextrin tea tree oil complex at WACKER's application lab: By molecular inclusion, various novel applications are possible – odor-free and without undesirable side-effects or a reduction in the effectiveness (*Photo*: Wacker Chemie AG)

moderate temperatures: its cavity is exactly the right size for the inclusion of effective tea tree oil components. As a result, a lipstick containing the β-cyclodextrin complex will not smell of tea tree oil even after several years' storage. The oil is not released until it makes contact with moist lips. The β-cyclodextrin tea tree oil complex is just as stable in wall paint. In the presence of moisture, paint containing this complex will continue to release the volatile oil for over four years.

The smaller α-cyclodextrin, comprising six glucose units, likewise turned out to be an excellent candidate for protecting the essential oil – provided that the guest molecule is enclosed by two α-cyclodextrin molecules. »*This 2:1 complex even withstands 60 minutes of heat treatment at 220 °C*«, explains *Regiert*. »*Enclosed in two α-cyclodextrin molecules each, the sensitive active-ingredient molecules are totally safe*«. This inclusion compound can be used, for example, to coat razor blades. During shaving, the blades release the antiseptic tea tree oil, thereby preventing the sensitive facial skin from becoming infected.

Cyclodextrins are based on starch. WACKER uses a biotech process that selectively produces the desired cyclodextrin. »*Inclusion within cyclodextrin is a promising and low environmental-impact technology that exemplifies the principle of sustainability*«, notes *Marlies Regiert*. Cyclodextrins have no known adverse health effects. Dermatologically speaking, there's nothing stopping their use in cosmetics, skin-care agents and personal care products or in surface coatings. Consistency is a further benefit of the inclusion compounds marketed by WACKER FINE CHEMICALS under its CAVAMAX® brand: they are colorless, odorless and free-flowing powders that are easy to store, handle and process.

The Background to Cyclodextrins

Cyclodextrins are non-reducing chiral sugars, whose molecules are made of several glucose building blocks linked into a ring. According to the number of glucose units – and therefore the ring size – a distinction is made between α, β and γ-cyclodextrin. α-Cyclodextrin has six, β-cyclodextrin seven, and γ-cyclodextrin eight glucose units. Cyclodextrins are natural degrada-tion products of starch. WACKER FINE CHEMICALS produces cyclodextrin from phytomaterials by a bio-engineering method.

In the cyclodextrin molecules, the glucose building blocks are arranged so that they have a lipo-philic cavity (i.e. one with an affinity for fat) in their interior. This cavity can receive another lipo-philic molecule as "guest," provided that it has the correct size and shape. The cohesion be-tween the two molecules is relatively weak (van der Waals forces), so that the guest molecule can be liberated again under suitable conditions. The weak van der Waals forces in such inclu-sion compounds leave the two counterpart molecules unchanged.

This ability to enclose other substances reversibly makes cyclodextrins invaluable in many pro-ducts and industries, such as household and personal care, pharmaceutical and cosmetic preparations, textiles and foods.

Fig. 3 The molecular inclusion in cyclodextrin stabilizes the ethereal oil, reduces the strong odor and enables a controlled release on the skin in juvenile form. This makes possible new applications that were previously unthinkable (*Photo*: Wacker Chemie AG)

New Impulses for Cosmetics – Wrapped up in Sugar Molecules

This enables the cosmetics and consumer goods sectors to take advantage of tea tree oil's entire spectrum of activity – without having to worry about undesirable side-effects, a loss of effectiveness or the intrusive smell of the free essential oil. The gates are wide open for totally new applications.

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Reduction of Irritations Caused by Household and Home Care Products

Abstract

Household, homecare products are commonly used in every field of our life. Apart of many advantages, there are plenty negative effects associated with use of homecare products. Especially skin is exposed to their undesirable influence, e.g: during hand-washing, dishwashing or in hard surfaces cleaning. It can lead to many undesirable skin reactions, such as: erythema, itching, oedema or splitting the dry skin. All mentioned factors are symptoms of contact dermatitis. Homecare products, especially their main ingredients: surface active agents may be associated with skin irritation and sensitization. They are directly responsible for the skin immune system response. They as well may decrease the skin barrier functions. A contact of surfactants with the skin may lead to hydro-lipid skin mantle removing from the skin's surface and may increase the transepidermal water loss (TEWL). That is the reason why homecare products cause also the changes in the intercellular cement »structure« and what follows, makes it easier for penetration of exogenous irritants. Because of that, nowadays household products should not only be effective in different kind of dirt removing, but also should have good dermatology properties.
In modern products from this market segment different steps are done to makes them effective and safer for consumers. During developing of new household product formulation mixture of different types of surfactants are used, therefore the critical micelle concentration (CMC) is lower and so irritant potential is decreased as well. Additionally the usable properties increase, e. g. foam stability. Another way to reduce irritation potential of homecare products is adding lipophilic agents (acting as refatting ingredients) or protein hydrolyzates. They prevent the disruption of the barrier function of stratum corneum. The second group may also decrease interaction of free molecules of surface active agents with proteins in epidermis as well as with s.c. intercellular lipid structure. Household, homecare products formulated in such way have lower skin irritation and corrosivity potential.

Introduction

The branches of chemical industry which are home care and household products, at recent year developed very supply. Products which help keeping our environment clean, good looking, shinning, conserved and with pleasant smell ere included to following group. Thise development goes hand with more and more often skin diseases, such as allergy and contact dermatitis. Increased TEWL, oedema, erythema or redness are typical symptoms of these unpleasant changes. This is caused by almost constant contact our skin with detergents. Environments, where we work and live, clothes that we wear are full of remains of these products. Thus we have observed very strong influence of household and home care products on the skin barrier functioning. The epidermis performs the main barrier function against the external and endogenous influence. Skin exposing for the exogenous factors, home care products, climate condition, UV radiation, cosmetics, can damage the proper function of the epidermis (1). As the scientists suggest and research confirm, there are strong relationship between the level of urbanization and industrial develop and frequency of skin allergy and sensitization (2).
In sum, beyond many advantages connected with household products usage, often negative effects may occur when they have direct or indirect contact with our skin. That is why manufacturers while creating their products, pay attention for good cleaning and washing qualities as well as skin care. The ingredients used in formulation should have high usable properties and should cause minimum side effects. Reduction of these effects is possible by several procedures. Products which have direct contact with skin may contain substances which will take care of skin surface and will strength the skin barrier function.

Household and Home Care Products

Basic components

These days there are about 40 categories of home care and household products. However basic components of them are similar. So called fundamental components include: surface active agents, acids or basis, solvents, dispersants and complexing agents, also special additives and fillers, such as bleaches, enzymes, fragrances, preservatives and many other. Usages of these products are determined by concentration of these ingredients, special additives, pH and way of applica-

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Property	Effects	Suitable Surfactants (example)
High synergic potential of surfactants and other ingredients	Improved performance profile reduction of the amount of surfactant required improved formulations	Linear alkylbenzenesulfonate, fatty alkyl ethoxylates, fatty alkyl ether sulfonates, alkyl polyglucosides
High wetting efficiency	Support of the cleaning performance; good run-off behavior, fast drying	Short-chain sulfonates
Foaming	High: hand-dishwashing liquids Low: detergents for dishwasher	fatty alkyl ether sulfonates end-capped fatty alkylethoxylates
Good solubility	Concentrates easier to produce reduction in or exclusion of solubilizing agent (hydrotropes)	alkanesulfonates
Favorable behavior in the cold	Low turbidity points	alkanesulfonates
Compatibility with electrolytes	Insensitive to water hardness	Nonionic surfactants
Chemical stability against alkalis, acids, chlorine	Formulations possible with very low and very high pH values or with oxidizing agents (including hypochlorite)	Sulfonate surfactants Stability against chlorine: amine-*N*-oxides

Table 1 Demands on surfactants, adopted from (3)

tion. Surfactants are categorized, in four groups: cationic, anionic, amphoteric and nonionic. The type of final product is determined by utilization and concentration of surfactants. The demands on surfactants are showed in **Table 1** (3).
Synergism of surfactants action has influence on final products properties, as well as on the viscosity, foam amount and stability. Mixture of surface active agents has other advantages, i.e. reduced critical micelle concentration (CMC). In case of final products which are characterized by lower concentration of surfactants monomers, irritant potential will be reduced (4-7).
Other important issue is pH of detergents. Depending on their purpose they may have different value, from strongly acid through neutral to basic. Relationship between pH and different kind of soil removal is given in **Fig. 1** (2).
PH value of detergents may have strong influence on proper functioning of skin barrier. Strongly acidic or alkaline products, which have direct contact with skin, may cause hydro-lipid skin mantle removal. It leads to increase of the transepidermal water loss (TEWL). Skin

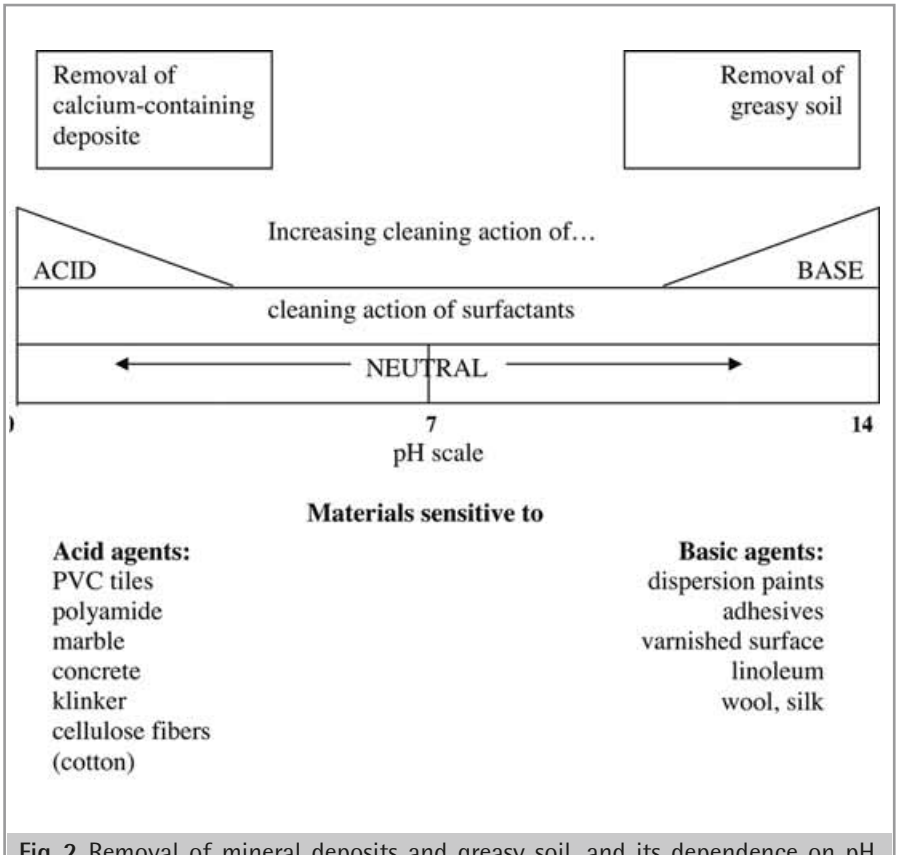

Fig. 2 Removal of mineral deposits and greasy soil, and its dependence on pH, adopted from (2)

exposure to these products, may also cause changes in proper intercellular cement architecture, which makes it more permeable for allergens (8).

Influence on the Skin

Home care and household products that have contact with our skin, for example during hand washing dishes or even when we wear cloths, from which detergents wasn't washed away completely, may have negative influence on the outer most layer of the skin. They often cause changes in epidermis barrier architecture and they lead to skin sensitization and allergy by two different pathways. Some ingredients of these particular products directly cause the skin immune system response, other lead to changes in structure of intercellular cement what can cause dryness of the skin. As a consequent of barrier structure alteration, potential irritants are able to permeate more easily and cause inflammatory reaction **(Fig. 2)** (9).

Surface active agents which are the main ingredients of these products, usual have very high irritant potential. Basically it depends on such features as chemical structure, concentration and value of free molecules of surfactants. To negative influences on the skin such actions can be included as: solubilisation and washing out lipids from the stratum corneum (this changes it's architecture and physicochemical properties) and damaging protein structure. As a consequent, transepidermal water loss (TEWL) increases as well as substances with high irritant potential permeability, which are present not only in household products, but also other products which have direct contact with »damaged« skin (7, 8, 9, 11).

Proper functioning of skin barrier is significantly influenced by extreme pH value of some detergents. Strong acid or alkaline pH may lead to hydro-lipid skin mantle removal. It has a disturbing effect on the proper build of horny layer. All these factors decreases skin barrier functioning (8).

Substances such as NaOCl, hydrogen peroxide or enzymes, which are added to household products for so called »hard spots« removal, may dry out the skin and lead to sensitization and allergy (12, 13).

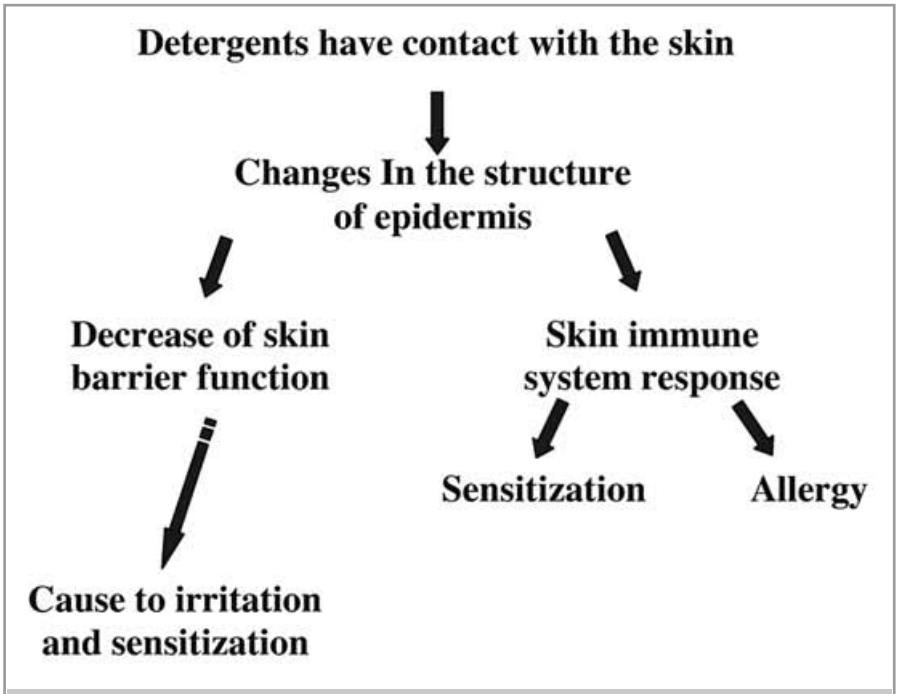

Fig. 2 Influence of homecare products on the skin, adopted from [10]

Method of Decreasing Irritant Potential

As mentioned before, the major aim of homecare and household products manufacturers is constant refining their products. They are looking for new ingredients, as well as new technologies to raise the quality of final recipes. They make their way towards creation of effective formulations which are mild to the skin at the same time. Side effects during direct contact with the products should be reduced. At the same time biodegrability of detergents and its ingredients is also important factor.

To reduce irritant potential of homecare and household products several procedure are possible. One of them is usage of surfactants characterized by good application properties and low irritant potential. Otherwise most often mixtures of surface active agents are used. The mixtures of these substances have lower irritant potential than the single one (7, 14).

In patch test, four hour skin exposure on 20% solution of sodium salt of lauryl sulfate (INCI Sodium Lauryl Sulfate) cause erythema, while en exposure to the mixture that contain, 20% Sodium Lauryl Sulfate, 10% Sodium Laureth Sulfate and 10% Cocodiethanoloamine, leaves a less intensive skin redness. Similar, lower irritant potential was noted for the mixture of Sodium Lauryl Sulfate and Lauroyl Glutamate (7).

Knowledge of skin irritation and corrosivity for single substance is well known and was confirmed by numerous *in vivo* and *in vitro* studies. However, simple summing up the irritant potential characteristic for individual surfactant is not sufficient for evaluation of concrete values of this parameter for its mixture. To con-

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firm that series of tests were done. 4 hr human covered patch tests, with combination of linear alkylbenzene (LAS), lauryl ether sulfate (SLES) and ethoxylated nonionic surfactant at 40% concentration were investigated. Results revealed that this mixture had lower irritant potential than control which constituted 20% solution of LAS (4).

Reduction of irritant potential for mixture of surface active agents is connected with lower CMC value. For combination of surfactants CMC is lower than for single one. It is correlated with number of free monomers in solution, which is responsible for negative effects on the skin. In **Fig. 3** and **4** patch test result are shown. Irritation is correlated with critical micelle concentration (CMC) value for individual surfactants and its mixture (4).

Studies described above show correlation and strong relationship among irritation potential and the value of the critical micelle concentration. This is the truth, but not for all cases. When we consider CMC for cationic and anionic surface active agents, similar values have cetrimonium bromide (CTAB) and SDS, however the irritant potential of CTAB is considerably much higher (4).

Among surface active agents, which are applied in various kinds of powders and liquids for washing, cleaning of various surface or liquids for dishes washing, anionic surfactants are applied the most often. Beyond good usable proprieties these substances are characterized by high irritant potential. As studies have shown, it is several times higher than for non-ionic surfactants and their derivatives. Thus using their mixtures with combination of Sodium Lauryl Sulfate is crucial for irritant potential reduction. Another advantage is their biodegrability (15).

Similar to alkylpolyglucoside (INCI: Laury Glucoside, Laurylglucoside Carboxylate), sulfosuccinates or betaines and their derivatives show low irritant potential and they might be used in homecare and household products in mixture with ionic surfactants. Additionally betaines show high stability in the wide range of pH value (16).

Generally, amphoteric surfactants are gentle for skin and eyes, characterized by low toxicity, high solubility in water, and also have an influence on foam stabilization and on the viscosity of solutions. They can be applied in preparations to washing and cleaning because of their low potential in disturbing skin barrier functioning (5).

Other way to lower the irritation potential of the homecare and household products is re-fatting substances addition. These ingredients have positive effect on skin barrier; for example during direct contact with these products, they prevent the disruption of the horny layer barrier functioning. They contain lipophilic substances, which reduce the inconvenient effect of the surfactants. (6, 14) Beyond this re-fatting substances used in washing liquids and powders have positive influence on sensory pro-

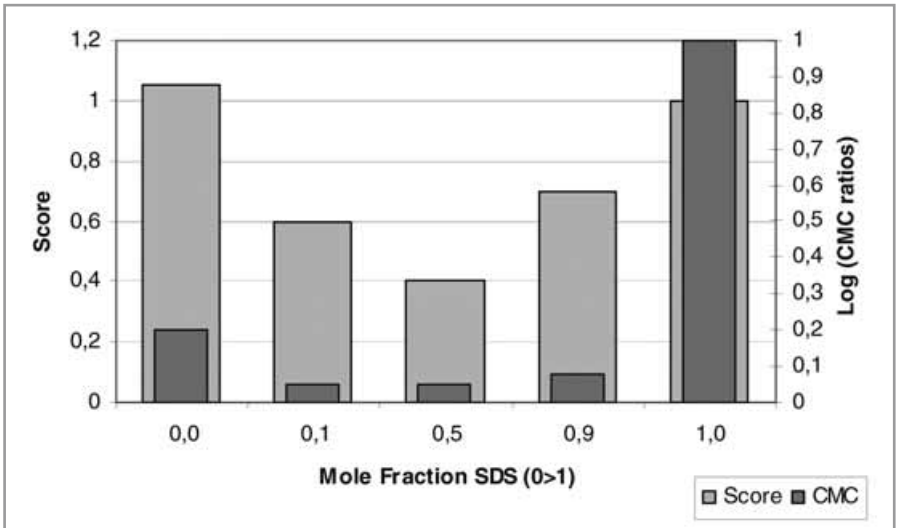

Fig. 3 Irritation score and CMC ratios v. mole fraction – DRAB (dimethyl dodecyl amido betaine)+SDS (sodium dodecyl sulfate), irritation is correlated with critical micelle concentration (CMC) value for individual surfactants and its mixture adopted from (4)

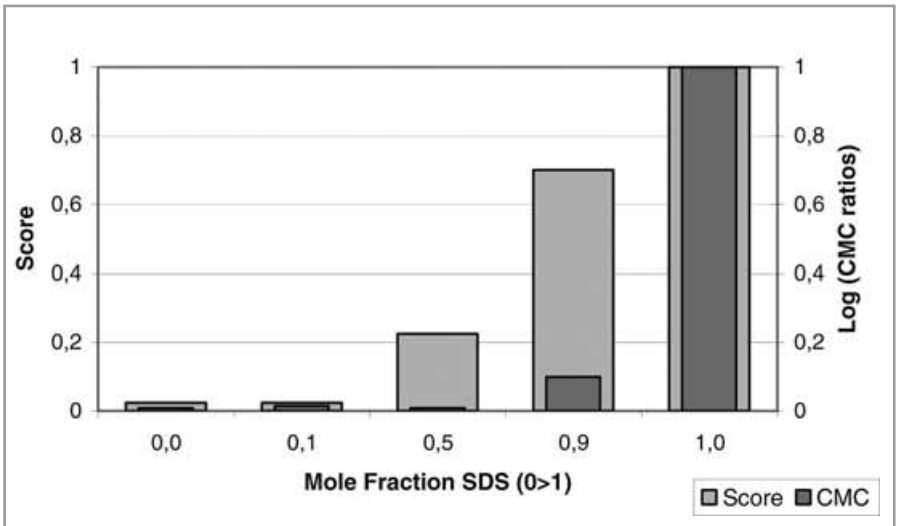

Fig. 4 Irritation score and CMC ratios v. mole fraction – APG (alkyl polyglucoside) +SDS (sodium dodecyl sulfate), irritation is correlated with critical micelle concentration (CMC) value for individual surfactants and its mixture adopted from (4)

file of fabrics. The subjective opinion people how were these clothes were decidedly higher. It was confirmed that re-fatting substances had the relation to fabrics, they reduce the friction among fibers and what follows elastics and smoothness are higher (6).
A similar positive effect on the skin have also the protein hydrolyzates, which are more often added to household products, especially to cleansing powders and liquids for children and people with sensitive skin. These substances have the strong affinity to the protein of epidermis, strengthening its barrier propriety, as well as taking care and preventing the skin from drying out. Additionally protein hydrolyzates, as substances with high molecular weight, decrease interaction of surface active agent monomers with proteins in epidermis as well as with s.c. intercellular lipid structure (14).

Summary

The irritant potential of homecare and household products can be reduce in many different ways that was described above. In practice, most often the compilations of these several methods are used. Next to mixture of surfactants with good usable and dermatology properties the protein hydrolyzates and re-fatting substances are added. To re-fatting substances are numbered fashionable recently lanolin and it derivatives and trend if we are talking about protein hydrolizates used in household products have various molecular weight.
It is very important, especially for producer but also for consumer to create formulation mild to the skin, because the recipe's compatibleness with the skin can be crucial factor examined by the consumers, particularly with such a broad offer in the market.

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AND EUROPEAN DETERGENTS CONFERENCE

OCTOBER 14–16 2009 | CONGRESS CENTRUM WÜRZBURG

PROGRAM

Wednesday | October 14, 2009 | Franconia Hall

Uhrzeit | Time	Title	Sprecher | Speaker	
09:00 – 09:30	**Conference commencement speech and greeting**	Prof. Dr. U. Buller, Prof. Dr. Th. P. Knepper, Fr. Bürgermeisterin Marion Schäfer	DE

Efficiency improvement of detergents and cleaners in view of sustainability

Moderation: Prof. Dr. U. Buller

Uhrzeit | Time	Title	Sprecher | Speaker	
09:30 – 10:00	**Keynote Lecture:** **Innovation instead of recession**	Prof. Dr. Thomas Müller-Kirschbaum	DE
	SEPAWA Subsidiary Award Ceremony	Prof. Dr. U. Buller	DE
10:00 – 10:30	Coffee break		
10:30 – 10:55	ADW Market – A reflection on the future from a supplier perspective	R. Parker, *H. Kohm, S. Razac, T. Tanakai*	EN
10:55 – 11:20	The Influence of Eco Design on Ingredient Choice in Home Care Products	Steve Johnson	EN
11:20 – 11:45	Saving Energy and Time: The Potential of Enzymes for Cold Water, Short Cycle Washing	O. Spangenberg	EN
11:45 – 12:10	Energy savings by low temperature washing	Prof. Dr. Rainer Stamminger, *Franziska Janczak, Dr. Dieter Nickel,* *Dr. Horst-Dieter Speckmann,*	EN
12:10 – 12:35	New enzyme takes ingredient substitution to a higher level	Peter Skagerlind	EN

The 56th SEPAWA Congress with European Detergents Conference with more than 45 scientific lectures and over 100 poster booths, offers the ideal opportunity to communicate with colleagues and exchange ideas. More than 1500 participants are expected this year, which makes the SEPAWA Congress one of the largest expert congresses in this field. We wish all delegates a successful and pleasant stay in Würzburg.

- Commencement and Formal Address
- SEPAWA Congress
- European Detergents Conference
- DGP Event

- DE Lecture Language German (Simultaneous Translation into English)
- EN Lecture Language English

Wednesday | October 14, 2009 | Barbarossa Hall

Modification of Surfaces

Moderation: Dr. Felix Müller

Uhrzeit | Time	Title	Sprecher | Speaker	
10:30 – 10:55	**GDCh-Specialized Group Detergent Chemistry Subsidiary Award Ceremony in the field of basic research of detergents and cleaners**	**Prof. Dr. Th. P. Knepper, Vorsitzender der GDCh Fachgruppe Waschmittelchemie**	DE
10:55 – 11:20	Fabrication and characterisation of biomimetic, hierarchical structures	Dr. Kerstin Koch	EN
11:20 – 11:45	Surface modification using stimuli-responsive polymer brushes	Prof. Dr. Wilhelm Huck	EN
11:45 – 12:10	AFM based Single-Hair-Force Spectroscopy for Optimizing Hair Care Products	Prof. Dr. Andreas Fery, *Eva Max, Claudia Wood, Frank Bartels, Albert Sugiharto,*	EN
12:10 – 12:35	Functionalization technologies for textile surfaces	Prof. Dr. Eckhard Schollmeyer	DE
12:35 – 14:00	Break & poster discussion		

Wednesday | October 14, 2009 | Franconia Hall

Efficiency optimization of detergents and cleaners through use of improved ingredients, optimized assessment process and responsible consumption behavior

Moderation: Dr. L. Möhle

Uhrzeit | Time	Title	Sprecher | Speaker	
14:00 – 14:25	New Silicone-Based Granulated Products for Home Care Applications	Kathelyne Everaere, *Jacqueline L'Hostis*	EN
14:25 – 14:50	"Clear view into a Green Future" – A hybrid polymer for hard surface cleaning	Marco Michaelsen, *Dr. Thomas Albers, Dr. Christoph Schunicht, Jeff Huh*	DE
14:50 – 15:15	Carboxymethyl Inulin: A multifunctional ingredient for eco-friendly detergents	Yves Boland, *Genevieve Bonnechere*	EN
15:15 – 15:45	Coffee break		
15:45 – 16:10	Washing and detergent dosing practise in German households	Sandra Bichler, *Elke Wieczorek, Rainer Stamminger*	EN
16:10 – 16:35	Comparison of the several standards for testing dishwashers or detergents	Anna Brückner, *Sabine Bornkessel, Anke Kruschwitz, Rainer Stamminger*	EN
16:35 – 17:00	Status of REACH Dossiers for Surfactants from the Lead Company Perspective	Frank Wangemann, *Konrad Gamon, Andreas Willing*	DE

Further Events

17:00	**Exhibitors' Info Event** for the SEPAWA Congress 2010 in Fulda
18:00	**Annual General Meeting of the SEPAWA** in the Franconia Hall

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Wednesday | October 14, 2009 | Barbarossa Hall

European Detergents Conference

New Compounds for Detergents: Structures and Properties

Moderation: Prof. Gradzielski, Prof. Laschewski

Uhrzeit | Time	Title	Sprecher | Speaker	
14:00 – 14:25	Physical and Chemical Modification of Polymeric Surfaces	Prof. Dr. Michael R. Buchmeiser, *R. Schubert, Ch. Elsner, A. Pender, L. Prager*	EN
14:25 – 14:50	Hair, cars, textiles: In quats we care	Dr. Joachim Venzmer	EN
14:50 – 15:15	Dynamics of Morphological Transitions in Self-Aggregating Systems with Non-Ionic Surfactants Studied by Stopped-Flow Experiments	Dipl.-Chem. Anina Barth, *S. Prévost, M. Gradzielski, I. Grillo, T. Narayanan*	EN
15:15 – 15:45	Coffee break & poster discussion		
15:45 – 16:10	Starch based thickening agents for personal care surfactant systems	Dipl.-Chem. André Lehmann, *Dr. Bert Volkert, Dr. Andreas Schrader, Dr. Heiko Nerenz*	DE
16:10 – 16:35	H*Proteins from BASF – modification of surface energies by new amphiphilic proteins based on Hydrophobins	Ulf Baus, *Dieter Boeckh, Thomas Subskowski*	EN
16:35 – 17:00	Self-aggregation of binary surfactant mixtures of dialkyldimethylammonium	Prof. Dr. Véronique Nardello-Rataj, *Gaétan Rauwel, Loïc Leclercq, Jean-Marie Aubry*	EN

Further Events

17:00 – 17:30	**Annual General Meeting of the "GDCh Fachgruppe Waschmittelchemie"** in the Barbarossa Hall
17:30	Discussion about the Scientific Poster Session in the Barbarossa Hall

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Thursday | October 15, 2009 | Franconia Hall

Conceptually effective ingredients and product strategies for cosmetics

Moderation: Dr. Ch. Kandzia

Uhrzeit | Time	Title	Sprecher | Speaker	
08:30 – 08:55	Keynote Lecture: Innovation in 'Green': Novel Microemulsion Technology for 'Natural' Skin and Hair Cleansing	Dr. Iris Hütter, *Guadalupe Pellon, Dr. Hans-Martin Haake, Dr. Hermann Hensen, Dr. Matthias Hloucha, Marc Beuché*	DE
08:55 – 09:20	Biocompatibility assessment of inorganic nanoparticles relevant for cosmetics	Andrea Salcher, *Vesna Aleksandrovic, Horst Weller*	EN
09:20 – 09:45	Advances in Stabilization of Formulations and Fragrances	Dr. O. Reich	EN
09:45 – 10:00	Coffee break		
10:00 – 10:25	Skin penetration ability of active substances from plant extracts	Anna Oborska, PhD	EN
10:25 – 10:50	Cellular test systems of the skin and real-time monitoring of cells as an alternative to animal testing	Dr. Michaela Noll	DE

Influence of the financial and economic crisis on the market situation

Moderation: Dr. K. Henning

Uhrzeit | Time	Title	Sprecher | Speaker	
10:50 – 11:15	How is the financial and economic crisis affecting the markets for detergents and home and personal care products?	Dipl. Kfm. Wolfgang Twardawa	DE

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Thursday | October 15, 2009 | Barbarossa Hall

LUV/HAD Session | Legal guidelines, environmental relevance and sustainability of detergents and cleaners

Moderation: Dr. R. Schröder, Dr. A. Rohrdanz

Uhrzeit | Time	Title	Sprecher | Speaker	
08:30 – 08:55	GHS: Effects on other legal areas	Prof. Dr. Herbert Bender	DE
08:55 – 09:20	REACH information transfer in the chain of delivery	Dr. Bernd Glassl	DE
09:20 – 09:45	Sustainable Consumption and Production: What does this mean form the viewpoint of the consumer?	Michael Kuhndt	EN
09:45 – 10:10	Coffee break & poster discussion		
10:10 – 10:35	Are detergents and cleaners safe for consumers?	Dr. Herbert Desel	DE
10:35 – 11:00	Requirements for ecological commercial cleaners in public requests for proposals	Marcus Gast	DE
11:00 – 11:25	What is a sustainable product?	Dr. Thorsten Wind	DE

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Thursday | October 15, 2009 | Franconia Hall

Formal Address

Uhrzeit | Time	Title	Sprecher | Speaker	💬
11:30 – 12:30	**Opportunities due to crisis – Crisis due to opportunities**	Prof. Dr. Utz Claassen	DE

Prof. Dr. Utz Claassen – Manager und Honorarprofessor (manager and honorary professor)
Aktuelle Tätigkeiten (current activities):
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Senior Partner, Valiance Capital/Valiance Infrastructure S.A., Luxemburg/Mailand
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Professor für Innovative Unternehmensführung, Risikomanagement und Wissensmanagement
an der GISMA Business School, Hannover

Thursday | October 15, 2009 | Barbarossa Hall

Raw materials for cosmetic formulations with special performance profile

Moderation: Dr. Vera Maienschein

Uhrzeit | Time	Title	Sprecher | Speaker	💬
14:00 – 14:25	Cosmetic efficacy in styling gels based on a new cationic thickener	Darshan Patwardhan, *Peter Hoessel, Joel Basilan, Bruno Gemba, Matthias Laubender*	DE
14:25 – 14:50	From Conditioning Shampoo to Nanomechanics and Sensory Experience on Human Hair	Claudia Wood, *Frank Wilco Bartels, Albert Budiman Sugiharto, Eva Max, Andreas Fery*	DE
14:50 – 15:15	Hair breakage – How to measure and how to counteract	Dr. Hans-Martin Haake, *Sandra Marten, Werner Seipel, Wolf Eisfeld*	DE
15:15 – 15:45	Coffee break		
15:45 – 16:10	A New Silicone Resin Wax Technology for High Performance and Innovative Aesthetics	Beatrice Durand	EN
16:10 – 16:35	Natural Moisturizing Factors and the Content of Water in the Stratum Corneum	Harald van der Hoeven	DE
16:35 – 17:00	Efficient Cationic Liquid Dispersion Polymer for Personal Care Concepts EO-free	Axel Böttcher, *Norbert Boyxen, Dr. Alexander Moll, Ian Lyons*	DE

Thursday | October 15, 2009 | Franconia Hall

DGP Session: Perfumery

Moderation: Gabriele Rehbock, Daniel Dillenseger, Dr. Wolfgang Krause, Dr. Dagmar Preis-Amberger

15:00 – 17:00	**DGP Session „The Spirit of Nature“**	Australian Sandalwood	Mr. Di-Lallo (Mount Romance)
		The Spirit of Nature – Expressed by its Scents	Prof. Roman Kaiser (Givaudan)
		No Compromise – Green Laundry and Home Care for the LOHAS Generation	Dr. Dreja (Henkel)

Die moderne Welt der Parfümerie basiert auf dem Zusammenspiel von Natur und Synthese. Wesentliche Anregungen für Produkte entstammen natürlichen Prinzipien, die dann in nachhaltiger Weise industriell umgesetzt werden. Das Programm „The Spirit of Nature“ behandelt dieses Themenfeld durch Beiträge aus Industrie und Wissenschaft.

Modern Perfumery often is a combination of nature and synthesis. Major product trends follow ideas and principles found in Nature. Sustainability has become an essential part of good corporate governance. This year's “Spirit of Nature” program encompasses both, scientific and industry relevant presentations.

Friday | October 16, 2009 | Franconia Hall

Cosmetic raw materials with specific property profiles

Moderation: Prof. Dr. U. Tannert

Uhrzeit | Time	**Title**	**Sprecher** | Speaker	💬
09:00 – 09:25	Comparative study on the influence of polymeric thickeners on the SPF of a sun screen formulation	Sonja Gehm	DE
09:25 – 09:50	Models for Simulation of Sun Protection Factors and Indices Characterizing the UVA Protection of Sunscreens: Principles and Applications	Dr. B. Herzog	EN
09:50 – 10:15	Dermatological studies of glutamate surfactants: Studies of pure substances and formulations suitable for natural cosmetics	Dr. Martin Husmann	DE
10:15 – 10:45	Coffee break		
10:45 – 11:10	Slowing down the cell proliferation – a new anti aging technology by using dormant plant extracts	Liki von Oppen-Bezalel	EN
11:10 – 11:35	Whitening meets Anti-Aging	Dr. Torsten Clarius, *Dr. Philippe Moussou, Dr. Vincent Bardey, Dr. Olga Freis, Isabelle Benoit, Dr. Gilles Pauly, Dr. Andreas Rathjens*	DE

Hybrid – Photostable UVA Protection

The European Commission's recommendation of 22 September 2006 is intended to ensure greater safety and transparency of sunscreens.
In future, sun protection products shall no longer only protect against sunburn, but also offer protection against all dangerous UV rays that cause premature skin ageing. Until recently, the cosmetics guideline has only included regulations on sunburn-inducing UVB rays. With the new recommendation, every sunscreen shall now also protect against UVA rays. These long-wave rays cause the skin to age prematurely, and possibly also impair the immune system. They are also considered a significant risk factor in the creation of certain types of skin cancer.
Now, there is also a new specification for the sun protection factor, where the UVA filter must be coupled to the UVB filter and must equate to at least one third of the UVB protection offered in the SPF.
This new regulation, which will probably become law in 2009, presents new challenges for the many cosmetics manufacturers who formulate sunscreen preparations or daily care products with sun protection.
Truly sufficient UVA protection can only be achieved using certain substances such as titanium dioxide, zinc oxide or the worldwide registered Avobenzone (INCI: Butyl Methoxydibenzoylmethane), also known as BMDBM.

Unfortunately, these products are not easy to formulate. Formulas with a high titanium dioxide content, for example, are always rated badly in terms of feel, transparency and spread; the approval status of zinc oxide is still a limitation; and it is well known that Avobenzone is not photostable.
The stability of Avobenzone is especially difficult to guarantee when conventional aluminium oxide coated titanium dioxides are used in formulas to achieve higher sun protection factors. The photostable combination with octocrylene so far patented has recently come under fire following new tests on this substance.
The saviours of the sun cosmetics formulator in this situation are the HYBRID UVA filters developed by SUNJIN.
The worldwide approved Avobenzone is encapsulated in polymethylmethacrylate (PMMA) together with octyl salicylate or 2-ethylhexyl 4-methoxycinnamate, and thereby protected against reactions with other ingredients and the resulting negative effects on the formula.
The texture additive PMMA isolates the UVA filter and has a positive effect on the feel of the formula **(Table)**.

Product	Composition		Particle size (µm)
Hybrid ABOS	BMDBM (Avobenzone) Octyl Salicylate PMMA (Polymethylmethacrylate)	25-30% 5-8% 62-70%	2-7
Hybrid ABOMC	BMDBM (Avobenzone) 2-Ethylhexyl 4-methoxycinnamate PMMA (Polymethylmethacrylate)	25-30% 5-8% 62-70%	2-7

Table HYBRID UVA filters

The advantages of the hybrid UV filter system:

- No potential for irritation
  The encapsulation reduces dermal resorption, thereby reducing the potential for irritation.
  Unlike organic UV filters, which penetrate into the skin and absorb the UV radiation, the contents of the hybrid systems rest on the skin's surface and form a practically invisible physical barrier against UV rays, comparable to the protective function of pigments. Irritation of the skin was tested using an *in vivo* patch test.

- Improved photostability
  The Avobenzone contained in the hybrids is so effectively isolated by the stable PMMA polymer matrix that contact between the UV filters – the cause of instability and odour problems – can be ruled out entirely.

- Easy working into formulas and improved formula stability
  The hybrids can be worked into the finished emulsion, while Avobenzone on its own has only limited solubility. The products can be used across a broad pH spectrum.

- Improved feel
  Many organic filters are very oily and leave behind an unpleasant, sometimes sticky feel on the skin.
  The hybrids have the excellent feel properties of PMMA, which is frequently used as a texture additive, and therefore lend the formula a soft-touch effect.

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< Content

Global Ingredients & Formulations Guide 2008

Sensory – Effects with Feeling

- **Beauty and Perception • New Actives**
- **Legislation • Formulations**
- **Raw Material Producers • Suppliers**

A German proverb says: **»You eat with your eyes«**. The meaning is, that humans evaluate products with more than one sense simultaneously. The optics, haptics, smell, origin, ecological aspects, economic facts, efficiency – all are important factors in the decision making of the consumer behavior generally and in the personal care industry especially.

To feel good means to look good. This simple truth is an important fact in the production and marketing of cosmetic products. It is therefore no surprise, that the sensory science affects all aspects of cosmetic research. This issue of the Global Ingredients & Formulations Guide describes extensively the various aspects and possibilities to improve sensory qualities of cosmetic products.

The GI&FG 2008 focusses on this subject (see content overleaf).

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Global Ingredients & Formulations Guide 2008 – Content

SolaStay™S_1 Outperforms Other Photostabilizers to Optimize UV Filter Performance

SolaStay™S_1, a groundbreaking new ingredient, can improve the performance of all types of sun protection products. When added to sunscreen formulations, SolaStay™S_1 can help sunscreen formulators to achieve previously unobtainable levels of protection against both the burning and aging rays of the sun.
»*Now personal care producers have a valuable new tool to formulate products that not only prevent sunburn, but protect against the aging effects of sun exposure at the same time*«, said *Craig Bonda*, Director of Research & Development for Personal Care Ingredients at HallStar.
When exposed to energy from solar ultraviolet (UV) radiation, UV filters like Avobenzone break down and lose their effectiveness. To prevent this, formulators add photostabilizers to their products to slow down the degradation. Sola Stay™ S_1 uses a different, better process to prevent or slow UV filter degradation, dramatically outperforming every other photostabilizer in the market. The result is maximized UVA protection and increased SPF.
SolaStay™ S_1 is suitable for lotions, creams, sprays, and gels **(Formulation)**. This is a powerful new tool for formulators who want UVA and UVB protection in their sunscreens and daily wear products (The HallStar Company, 120 South Riverside Plaza Suite 1620, Chicago, IL 60606, USA, Tel. 877 427 4255 (International callers: +1-312-385-4494), www.hallstar.com).

This globally acceptable, daily wear quality, formula yields superior broad-spectrum protection by using SolaStay™S_1, a novel photostabilizer that quenches the singlet excited states of Avobenzone and Octinoxate, and HallBrite®, an emollient and sunscreen solvent that adds a nice touch of elegance to the afterfeel.

Phase	Ingredient	INCI Name	% wt
1	Ethylhexyl Methoxycinnamate	Escalol® 557 (1)	7.50
2	Homosalate	HallBrite® HS USP (2)	10.00
3	Ethylhexyl Methoxycrylene	SolaStay™ S1 (2)	6.00
4	Butyloctyl Salicylate	HallBrite® BHB (2)	5.00
5	Tocopheryl Acetate	Vitamin E Acetate Oil USP, FCC (3)	0.20
6	Butyl Methoxydibenzoylmethane	Neo Heliopan 357 (4)	3.00
7	Aloe Barbadensis Leaf Juice	CoVera™ (2)	0.50
8	SD Alcohol 40		63.80
9	Acrylates/Octylacrylamide Copolymer	Dermacryl® (5)	2.00
10	Hydroxypropylcellulose	Klucel Hydroxypropyl-cellulose (6)	2.00

(1) ISP (2) HallStar (3) BASF (4) Symrise (5) National Starch (6) Hercules

Preparative Procedure
An explosion-proof environment is required throughut preparation and packaging.

Add 1 - 5 to secondary vessel, start center-stir (prop) mixing, heat to 50 °C and then add 6. When homogeneous and clear, start cooling.
To the primary vessel add 7 and 8. With mixing add 9.
Slowly add secondary vessel contents to the main vessel contents.
When uniform and clear slowly add 10 and mix for 20 minutes.
When uniform and clear, perform final quality assurance checks and package product in tube.

Product Characteristics (25°C)
Appearance: Clear yellow gel
pH: 6.5
Viscosity (RV, T-D, 5, 20 & 100 rpm; cps): 70000, 17300, 5160

Formulation Broad Spectrum SPF ~40, PFA (PPD) ~15 Sunscreen Gel

Wash-On™ Technology: UV Protection by Body Wash

Aquea SPF™ is the first fully functional, clinically proven, safety-tested Wash-On™ UVA/UVB sunscreen ingredient that can be added to soaps, facial cleansers, body washes, shower gels and shampoos. And because it is water based, that feeling of fresh-washed cleanliness is enhanced – unlike the oily residue that most sunscreen products leave on your skin.
What does Wash-On™ mean to you? Imagine taking a shower and being automatically protected by your Body Wash or Shower Gel over every part of your body. Imagine shampooing and having your scalp shielded from the harmful rays (hair does not block UV). Imagine merely washing your face and having it protected.
Aquea SPF™ is not meant to replace the SPF 15 or SPF 30 sunscreens you use under intensive sun. But Aquea SPF™ does help protect against both UVA rays (associated with wrinkles and aging) and UVB rays (associated with sunburns). When used as an ingredient in any Wash-On™ product, Aquea SPF™ affords enough sun protection for everyday use by leaving a virtually undetectable shield on the skin. It's a long-lasting shield because the double-layered positively charged microcapsules containing the sunscreen are attracted to our negatively charged skin like a magnet (Distributor for Germany, Austria, Switzerland, Slowenia, Serbia, Kroatia, Bulgaria, Czech Republic, Romania, Slovakia, Poland and Skandinavia: NRC, Nordmann, Rassmann GmbH, *Oliver Koopmann*, Tel. +49 40 3687384, Email: info@ nrc.de, www.nrc.de).

Melatime™ for Faster and Longer Lasting Tan

Melatime™ is a novel peptide that boosts the tanning process of the skin exposed to UV radiation. Moreover, this new product prolongs the tan after two weeks of the last irradiation, providing a long-lasting effect. Melatime™ is a palmitoyl tri-peptide that stimulates cAMP production (cyclic adenosine-3'5'-monophosphate) increasing melanin synthesis and accelerating the tanning process under UV-inducing conditions.

In vitro efficacy
Primary human epidermal melanocyte cells were incubated with Melatime™ at different concentrations. Vehicle was used as negative control and 40 µM Forskolin as positive control. Melatime™ increases by 180% the melanin content after 4 days of treatment.

In vivo efficacy
Melatime™ proved *in vivo* its accelerating and long-lasting tanning properties. It reduced by 108% the Individual Typological Angle (ITA)° values more than placebo in the first week of treatment under UVA- inducing conditions and 14 days after last irradiation showed a decrease of 40%.

Melatime™ provides a natural tan to the skin fitting the constitutive colour and maintains the tan two weeks since the last irradiation providing a long lasting effect (Lipotec, *Elena Cañadas*, Tel +34 93 638 80 00, Email: ecanadas@lipotec.com).

Peptan™ Proven for Moisturizing and Anti-Aging Effects

Over the last few years, Rousselot has developed unique qualities and grades of hydrolyzed collagens that are sold and consumed throughout the world under the Peptan™ brand.
To assess the efficacy and acceptability of Peptan™, Rousselot has commissioned two double-blind randomized clinical studies versus placebo in France and in Japan. The objective was to definitively provide nutricosmetic manufacturers as well as end consumers with reliable and accurate measurements of the benefits of Peptan™ Hydrolyzed Collagen for skin.
In France, 47 European women aged 35 to 55 were selected by DERMSCAN, to participate in a study over a 12-week period in winter. At the same time in Japan, 33 Asian women aged 40 to 59 were select by SOUKEN, for a period of 8 weeks.
In both studies, women ingested 10g of Peptan™ Hydrolyzed Collagen every day, and their skin conditions were assessed by dermophysiological measures and self- assessment questionnaire.
The first evaluation of Peptan™ was for its moisturizing effect. Measurements demonstrated a 28% improvement in the skin hydration level in the Peptan™ group compared with the placebo group. 91 % of Peptan volunteers skin hydration level increased after an 8-week test.
The second evaluation of Peptan™ was for its anti-aging effect. The number of micro-relief furrows decreased by 26% compared with the placebo group. The number of deep wrinkles increased by 30% with the placebo group while remaining stable within the Peptan® Group after a 12-week test.
Researchers conclude that when taken daily for up to 12 weeks, Peptan™ Hydrolyzed Collagen improves the basic condition and structure of skin: Peptan® has been clinically shown to improve skin moisture level, skin suppleness and skin smoothness by reducing the number of micro-relief furrows and prevent the formation of deep-wrinkles.
With the population tending to be increasingly conscious of the importance of good skin health, Peptan™ Hydrolyzed Collagen is indisputably a highly potent ingredient for the nutricosmetic market (Rousselot, *Caroline Brochard-Garnier*, Tel. +33 1 46 67 87 27, Email: caroline.brochard-garnier@rousselot.com).

La'Youth – Exotic Naturals for Ageless Skin

The fountain of youth and youthful skin can be achevied and maintained by using the vast and proven knowledge of Ayurveda, the world's oldest system of health care. Herbal Extracts, Fruit Extracts & Essential oils have been used effectively for thousands of years in Medicines, Food supplements and Personal Care by Ayurveda. Ayurvedic Cosmeceuticals are very much appraised for their holistic as well as their adverse free action. They are 100% natural, obtained from mother nature by maintaining the natural botanical intelligence of the plant.
La'Youth tonifies the skin, smoothes its imperfections, increases its hydration level, thus restoring a radiant and healthy look. It effectively reinforces the skin's self-protection against dehydration, oxidative stress and also degradation of the extra-cellular matrix, and stimulates the collagen biosynthesis. Results in an improvement of tone and elasticity of venous wall and reduction of abnormal capillary permeability. La'Youth improves firmness, smoothness while reducing imperfections, inflammation and sebum flow and actively protects the skin and prevents premature aging.
Lachemi Chemorgs offers a range of La'Youth products:

La'Youth A[a] - Age old anti aging secret of Ayurveda using exotic herbal, flower and seedextracts, etc.
(INCI: Crocus sativus, Rubia cordifolia, Nelumbo nucifera, Shilajit etc).

La'Youth SFE - Anti aging combination of extracts from fruit and flower seeds rich in antioxidants and vitamins and enzymes.
(INCI: Vitis vinifera, Daucus carota, Triticum vulgare, Calendulla officinalis, Moringa oleifera etc.)

La'Youth MOI - Moisturizer combination of extracts from fruit and flowers rich in omega fats, antioxidants, vitamins etc.
INCI: Rosa centifolia, Simmondsia chinensis, Prunus amygdalus var.dulcus, Oryza sativa, Cucurbita pepo etc.)

La'Youth VIT – Nutritive extracts providing all essential vitamins A, B Complex, C, E etc.
(INCI: Moringa oleifera, Triticum vulgare, Oryza sativa etc.

Lachemi Chemorgs (P).LTD, Tel. 91 20 2426 9118/24264965, Email: info@lacemi.com, www.lachemi.com.

Sun Care Solutions from Cognis Care Chemicals

Laboratoires Sérobiologiques (LS), the active ingredient business of Cognis Care Chemicals, offers a wide range of high quality actives with many unique benefits applicable to sun protection products. DN-AGE LS 9547 is a botanical complex, extracted from the leaves of Cassia Alata, recommended for the use in anti-age care, to protect cellular DNA against UV radiations. SUNACTYL LS 9610, a multi active botanical and biotechnological complex, is recommended for the use in anti-UV-stress preparations for the face, and sun care products. For manufacturers wishing to make multi-functional product claims, active ingredients are available from LS to support skin firming and slimming, sun tan maintenance, radiance enhancement or even anti-aging claims as well as a specific product range dedicated to very sensitive skin.

With consumers' increasing focus on wellness and a desire for healthy looking, the self tanning market has exploded over recent years with the introduction of daily tanning products. Cognis offers Ultragel 300 and Cosmedia CTH(E) which are both ideal for this product cate- gory. Ultragel 300 allows the formation of clear gels at the acidic pH necessary for self tanning products while Cosmedia CTH(E) is ideal for stabilizing lotions and creams at low pH.

Choosing the right emollient and emulsifier

When developing a sun care formulation, choosing the right emollient is particularly important, as it can enhance the sun screen solubility, pigment dispersion and SPF. Cognis offers a range of emollients which can be used individually or in combination to aid chemical sun screen solubility and disperse physical sunscreens. Cetiol CC offers an excellent dry, soft and silky skin-feel. Myritol 331 is ideal for »green« sun screen developments with its excellent natural profile. Cetiol B is an ideal solubilizer with great performance with some of the newer sunscreen fil-ters available **(Formulation)**. Cetiol SenSoft, the »happy emollient«, is the right choice for facial sun care with its truly luxurious skin-feel.

Another product in Cognis' emollient range is Cosmedia Gel CC, which smoothes the skin's surface, and has a melting texture with a velvety matt effect. It enhances the performance of sun care products, especially by improving water resistance. Cosmedia® Gel CC works as a suspending agent; it stabilizes emulsions, improves viscosity and thermo stability, and optimizes gel dispersion.

Cosmedia DC is an innovative water resistant agent from Cognis that helps the formulator to achieve water resistance whilst actually improving the sensory performance achieved. The »Switch Emulsion« technology provides fast water resistance and an additional moisture locking benefit. Cosmedia DC is compatible with ethanol; organic and inorganic filters can be used with emulsifiers of all kinds. The product makes it simple to achieve stable formulations.

Furthermore, emulsifier choice can make or break any sunscreen formulation. With Cognis' new generation of emulsifiers you can hold it all together without any negative impact on sensory performance. Cognis has recently launched Eumulgin Prisma, a high performance emulsifier which is compatible with high levels of water soluble sun screens and actives. For »green« sun care products their APG based emulsifiers come in liquid or solid form; Emulgade PL68/50 and Eumulgin VL75 (Cognis GmbH, Rheinpromenade 1, 40789 Monheim, *Raquel Ark*, Tel. (02173) 4995-464, Email: raquel.ark@cognis.com, www.cognis.com).

Phase	INCI Name	Ingredient	%
I	Cetearyl Glucoside (and) Cetearyl Alcohol	Emulgade® PL68/50 (1)	1.0
	Sodium Cetearyl Sulfate	Lanette® E (1)	1.0
	Cocoglycerides	Myritol® 331 (1)	5.0
	Dicaprylyl Carbonate	Cetiol® CC (1)	5.0
	Cetiol® OE	Dicaprylyl Ether (1)	3.0
	Ethylhexyl Methoxycinnamate and BHT	Eusolex 2292 (2)	7.5
	Octocrylene	Neo Heliopan 303 (3)	9.0
	Hydrogenated Dimer Dilinoleyl/ Dimethylcarbonate Copolymer	Cosmedia® DC (1)	2.0
	Zinc Oxide	Zinc Oxide	5.0
		Laponite XLG	2.0
II	Glycerin	Glycerin	3.0
	Water, dem.	Aqua	56.5

(1) Cognis (2) Merck (3) Symrise

Preparations in the Laboratory:
Heat phase I to 80 °C. Heat phase II to 80 °C and add to phase I while stirring. Allow the emulsion to cool while stirring in such a way that it remains in continual motion. Avoid incorporation of air.
If necessary, homogenize with a suitable dispersion unit (e.g. Ultra Turax) at approx. 55 °C.
Stir while cooling to 40 °C and add phase III (if available). Stop stirring at 30 °C.

Viscosity (mPas) (Brookfield RVT 23 °C spindle 5, 10rpm; 28,000

Formulation O/W Sun Fluid Spray

The Herboretum – An Association Supported by Two Acclaimed Names of the Cosmetics Industry

The story begins in 2004, when a dozen Nature-lovers, particularly aware of the environmental issues concerning their respective disciplines, decided to commit themselves, in favour of the preservation of biodiversity, and created the Herboretum Association. Their action field: The Herboretum, a garden of preserved and shared biodiversity, located in Saint-Ay, Val de Loire region, south of Paris, listed as UNESCO World Heritage.

The association and its project, which has been acknowledged as being of general interest, has been supported by the Alban Muller Group ever since its creation. The Alban Muller Group was founded more than 30 years ago. It explores the planet's extraordinary natural reservoir.

Today, it stands as an expert in plant-origin active principles and formulating top-quality natural products for the Beauty, Health, and Well-Being industries. In the context of its ecoresponsible philosophy, it was only natural for the group to commit to working hand in hand with The Herboretum.

It was not long before the Clarins Group joined the ambitious project. Clarins, the leader in the selective perfume industry in France and Europe, is naturally aware of the stakes in protecting biodiversity, and has favoured the encounter of science and nature, putting a lot of effort into protecting the natural heritage since its origin. It prizes plant origin compounds in all its products, respecting the environment and preserving the planet's resources. Supporting The Herboretum was thus an obvious step for this company, which is socially aware and committed to sustainable development.

The project developed thanks to the subsidiaries of these eminent names in the cosmetics industry, and to the know-how of its president and numerous experts, and it consequently led to a preserved and shared garden of biodiversity.

Today The Herboretum reflects, acts in favour of, and is an ambassador of biodiversity, developing a programme of activities focusing on 5 fundamental points:

- Preserving biodiversity, with environment-friendly cultivating methods
- Highlighting biodiversity, by presenting a variety of gardens
- Observing biodiversity, to further understand its complexity
- Participating in the follow-up of its evolution
- Making the public aware of its vital role

Publication of COSMOS-standard

BDIH (Germany), BIOFORUM (Belgium), COSMEBIO & ECOCERT (France), ICEA (Italy) and SOIL ASSOCIATION (UK) are pleased to announce the publication of COSMOS-standard, the new harmonized and most challenging European cosmetics organic and natural Standard. The final version of the Standard is available on its website www.cosmos-standard.org.

After six years of intensive work and an international public consultation of three months, the European Cosmetics Standards Working Group is pleased to release the final outcome with the publication of COSMOS-standard. The objective of this new Standard is ambitious and goes beyond the harmonization of minimum requirements for organic and natural cosmetics.

Recognising the challenges of maintaining the natural balance of the planet and the responsibility of the 1,000 certified companies they account for, the European group clearly shows its ambition to go further with the setting of an internationally accepted standard for organic and natural cosmetics. »*The long-term objective of COSMOS-standard is to actively contribute to establishing sustainable development by stimulating changes in production patterns and consumption practices*« says *Riccardo Anouchinsky* of ICEA. »*This implies promoting always more the use of products from organic agriculture, the responsible use of natural resources while respecting biodiversity and the environment*« added *Muriel Huybrechts* of BIOFORUM.

The market for organic and natural cosmetics is expanding and this is crucial for the benefit of all, from suppliers of raw materials, formulators to consumers to implement rules that enhance innovation. »*This is why COSMOS-standard sets as well requirements for processing and manufacturing that must be clean and respectful of human health and environment by integrating for the first time in a Standard the principles of Green Chemistry*« added *Betty Santonnat* of COSMEBIO.

»*The European group will now be working on harmonizing the key points of the subsequent control plan and we expect to be able to start certifying according COSMOS-standard on September 2009*« says *Valérie Lemaire* of ECOCERT. »Other certification/inspection bodies that are not members of the group will have the possibility to apply to use this Standard. The procedure for application and authorisation will be opened as from September 30th, 2009« added *Francis Blake* of SOIL ASSOCIATION.

»*With such an important development, we consider vital to involve all key players of the field and for this reason we will open the COSMOS-standard up to new members via organizations of raw material suppliers, cosmetic laboratories and manufacturers as well as certifications bodies*« concluded *Harald Dittmar* of BDIH.

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BASF Presents Concept for Basel Site

Effective July 1, 2009, BASF's Paper Chemicals operating division, which was established in April, will be headquartered in Basel, Switzerland, together with two associated business units: Coatings & Starch Europe and Wet End Chemicals. In addition, the European business unit for plastic additives and the global units for technology management and the restructuring of the pigment business will be located at the former headquarters of Ciba Holding AG in Basel. BASF is also establishing a new Business Center Switzerland in Basel, which will provide a platform for sales as well as finance and personnel functions in Switzerland. Basel will also be home to a BASF research center focusing on plastics, coatings and paper.
Following the closing of the acquisition on April 9, 2009, teams of experts from BASF and Ciba started analyzing the best possible combination of the businesses. The decision in favor of Basel underlines the strong position the former Ciba headquarters will have within the BASF Group. BASF said that Basel would be the headquarters of one of its global operating divisions when it announced its plans to acquire Ciba in September 2008. Detailed integration plans and the necessary restructuring measures will be published in July.
Dr. *Fred Baumgartner* was appointed head of the Paper Chemicals division effective April 2009. This division combines products and solutions to improve the production and properties of paper.
As planned, BASF intends to merge Ciba Holding AG with BASF Specialty Chemicals Holding GmbH, Basel. The remaining minority shareholders in Ciba will then receive a cash compensation of CHF 50 per share.

Cognis Opens Affiliate in India

Cognis has opened an affiliate in Mumbai, India. Cognis India will be a wholly-owned subsidiary of the Cognis Group, and will operate under the name of Cognis Specialty Chemicals Pvt. Ltd. Cognis first opened a liaison office in India in February 2008. The new affiliate will continue to grow Cognis' business in India, and highlights the importance of this region.

SEPPIC Joins »Cosmetic Valley«

SEPPIC has now joined the »Cosmetic Valley« competitiveness cluster. The world's leading cosmetics center, this group of companies offers a wealth of human, material and financial resources to support French businesses, helping them create innovative products and conquer new international markets.
By joining this cluster, SEPPIC will be in a position to benefit from the cross-fertilization of the cluster's skills, allowing it to reinforce its open innovation program, Sep'Innove.
The SEPPIC group's most recent launches include innovations inspired by nature and approved by ECOCERT:
SERENIKS 207 ECO, an active anti-ageing ingredient derived from Eastern hemlock (a coniferous tree), with a high resistance to cold to protect sensitive skin; SOLAGUM AX, an original combination of acacia and xanthan gum for a natural thickening polymer; ADIPOLESS ECO, a new active slimming ingredient derived from quinoa and ROSMARINYL GLUCOSIDE, polyphenols extracted from lemon balm, which are stable and water-soluble for improved cutaneous bioavailability.

DSM and Lonza Strengthen their Partnership

DSM Nutritional Products Ltd and Lonza Ltd announce the early renewal and extension of their long-time collaboration in the production facilities in Visp and Lalden, Wallis, Switzerland.
Lonza has for decades been a critical supplier for the services and raw materials for the Vitamins, Carotenoids, and aroma chemicals produced by DSM Nutritional Products. Within the framework of this new contract, the parties are reviewing the organisational setup for the 147 Lonza employees working at the Lalden site of DSM Nutritional Products. In today's environment it becomes of increasing importance to intensify partnerships in order to be able to innovate, deliver the highest quality, and secure future growth at competitive conditions. All these elements are intrinsically part of the partnership between Lonza and DSM Nutritional Products.
The collaboration between the companies dates back to 1965, when the predecessor company Teranol AG was founded to produce vitamin A and E. As Lonza commissioned at that same time its cracker, which could supply the necessary raw materials acetylene and hydrogen, the decision was made to locate the new Teranol production site in Lalden next to existing Lonza infrastructure.

Procter & Gamble: Susan E. Arnold Retires

Susan E. Arnold, president – global business units, will retire effective September 1, 2009, after 29 years of service, P&G announced. After stepping down as president she will continue to serve in a special assignment, reporting to Chairman of the Board and Chief Executive Officer A.G. Lafley, until September 1. »It has long been her intention to step down upon her 55th birthday, which she celebrated on March 8, 2009«, the company informed.
Most recently, *Susan* has played a pivotal role in P&G's focus on sustainability. Under her leadership, P&G adopted company-wide sustainability goals. Sustainability has been integrated into strategies and plans in every business unit. She has championed social sustainability and has sponsored P&G's Children's Safe Drinking Water program, which has delivered more than a billion liters of safe drinking water to people in need around the world.

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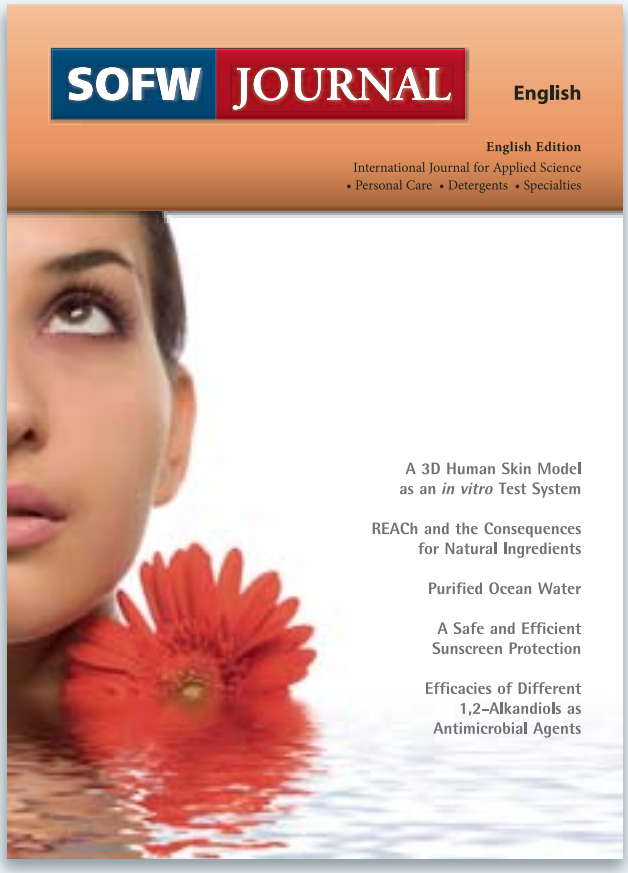

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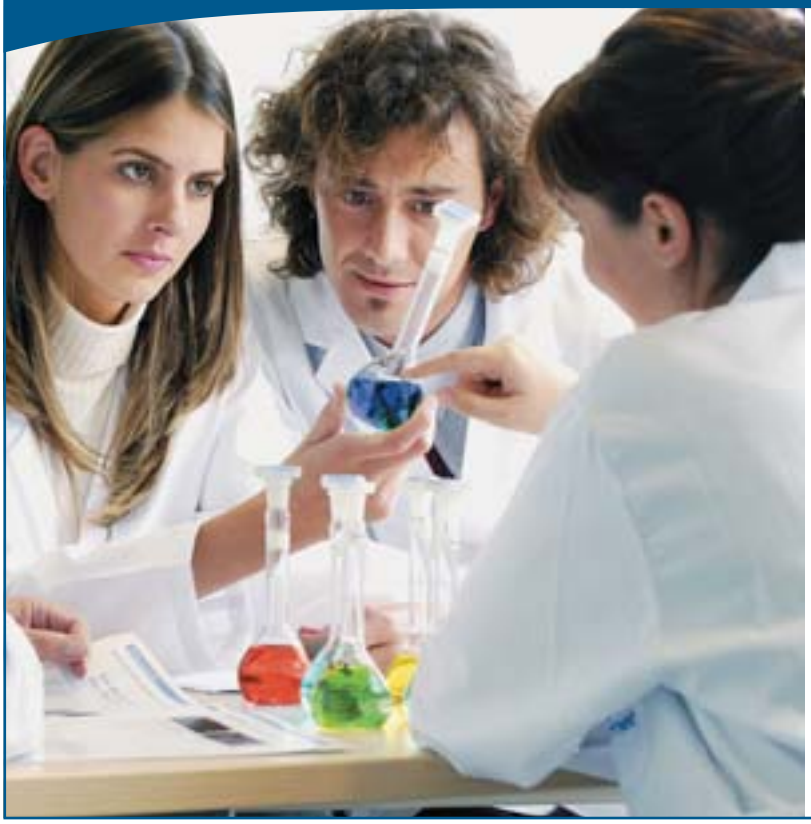

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