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Assessment of skin sensitizing potential of UV-filters by a novel *in vitro* method based on DPRA

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Raw materials

Most related to Allergic Contact Dermatitis

- Higher risks:

Nickel

Fragrances

Preservatives

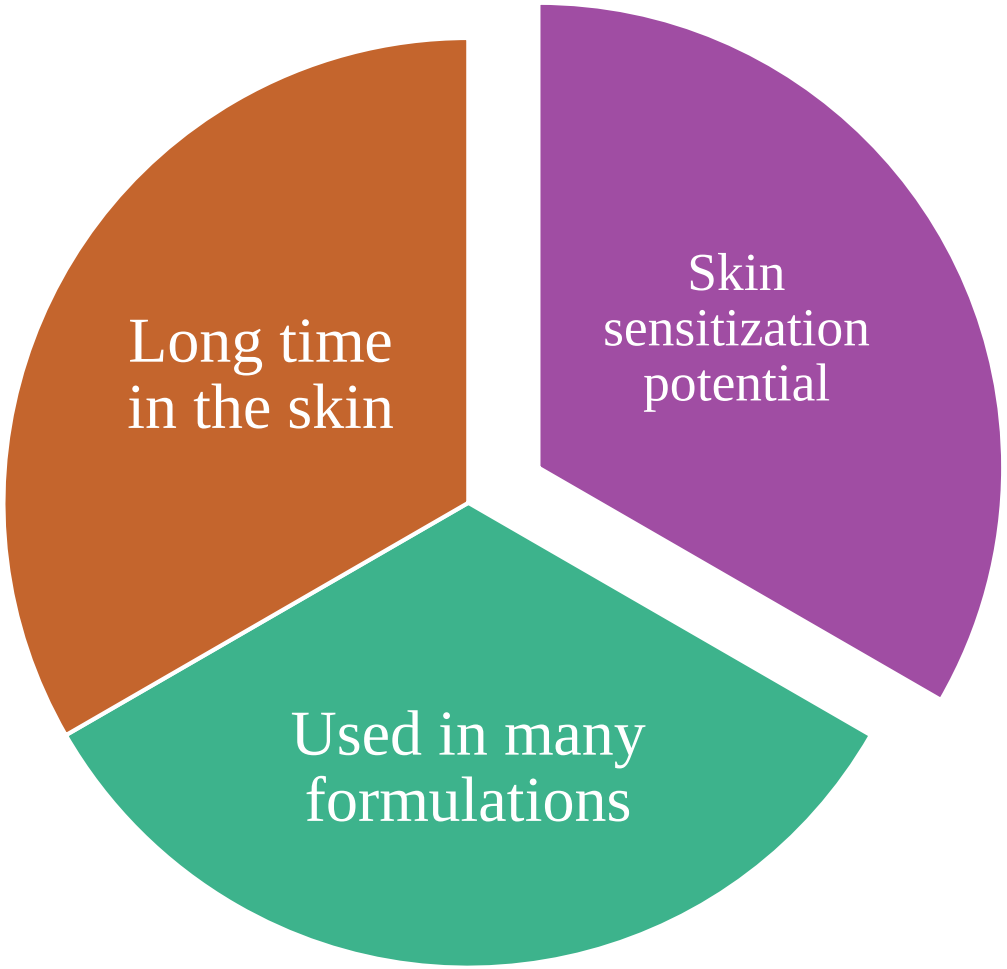
Hair dyes

UV-filters

https://www.medindia.net/news/healthwatch/allergic-contact-dermatitis-induced-by-cosmetics-86059-1.htm

(Thyssen et al., 2007; Avenel-audran, 2010; Nash, 2006; Saraswat, 2012; Tosti et al., 1991)

UV-filters



(Scheman, 2000)

Mechanisms associated with skin sensitisation

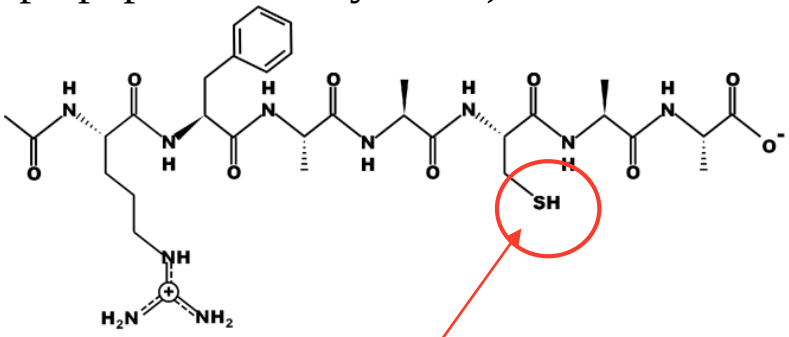
Adverse Outcome Pathway (AOP) and Alternative methods validated (key events):

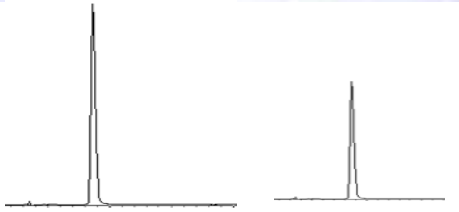
1) **Covalent binding** of electrophilic substances to nucleophilic centres in skin proteins. **(DPRA 442C)**
2) **Keratinocytes**: inflammatory responses and changes in gene expression associated with specific cell signalling pathways - (ARE)-dependent pathways. **(442D: KeratinoSens, LuSens)**
3) **Activation of dendritic cells (DC):** expression of specific cell surface markers, chemokines and cytokines. **(442E: h-CLAT, U-SENS™, IL-8 Luc assay)**
4) **T-cell activation and proliferation:** murine Local Lymph Node Assay **(LLNA 442B)**

Limitations: lipid soluble compounds

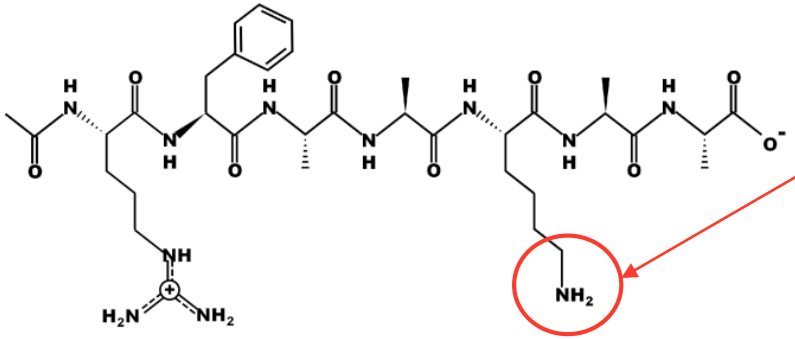
DPRA – proteic haptenization in vitro

Ac-RFAA**C**AA-COOH
(heptapeptide with cysteine)





Ac-RFAA**K**AA-COOH
(heptapeptide with lysine)



Nucleophilic heteroatoms

covalent binding of electrophilic substances to nucleophilic centres in skin proteins

(Gerberick et al., 2004, 2007, OECD TG 442C)

Objective

Present a modified DPRA test, which uses synthetic peptide analogues containing hydrophobic amino acids, which are able to react with electrophilic and more lipophilic chemical allergens (haptens).

Methods

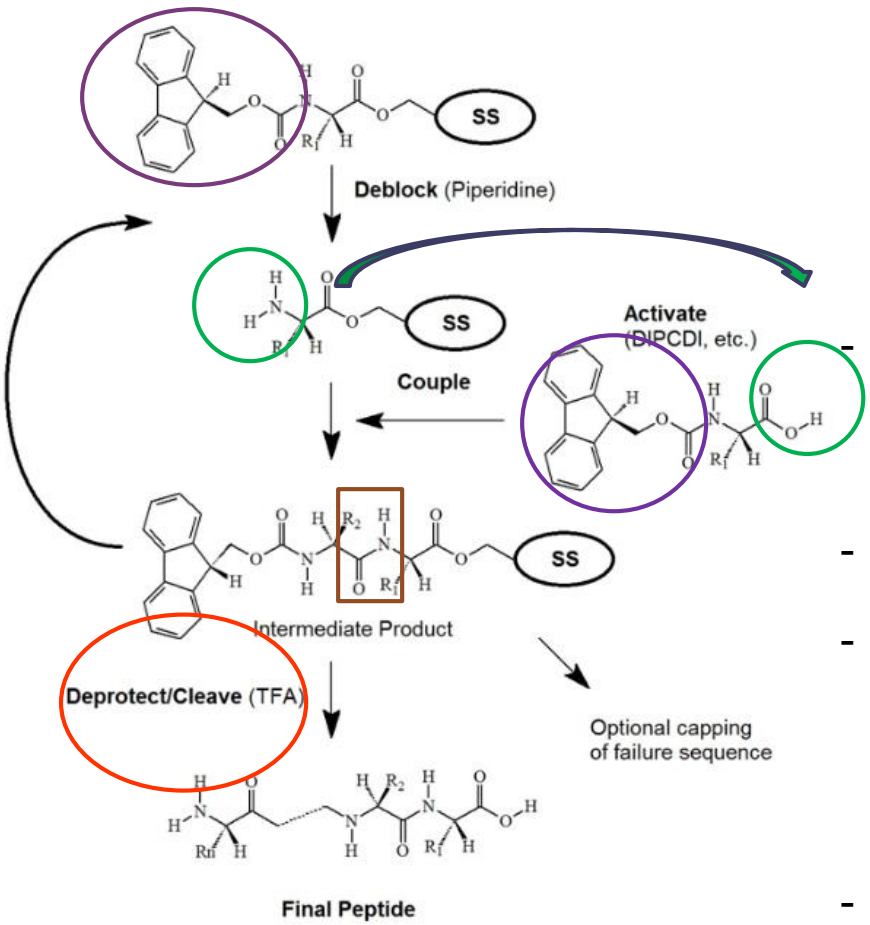
Cysteine based heptapeptides modifications

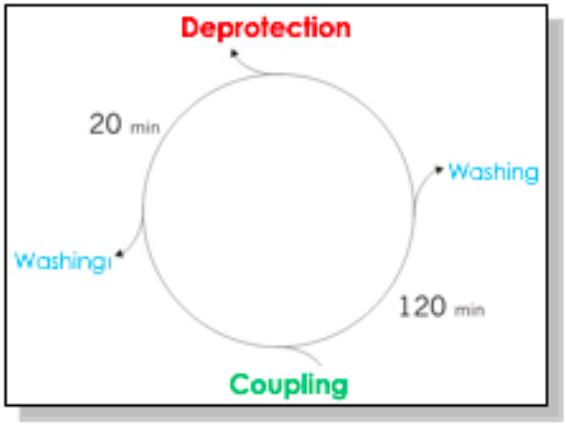
	Proposal
Original peptide (CIS)	original peptide containing **Cysteine**
Modification 1 (peptide FVC)	Replacement of Ala by Val to enhance peptide lipid solubility
Modification 2 (peptide WAC)	Replacement of Phe (F) by Trp (W) to allow detection at 280 nm
Modification 3 (peptide WVC)	Replacement of Ala by Val to enhance peptide lipid solubility and Phe (F) by Trp (W) to allow detection at 280 nm
Modification 4 (peptide YAC)	Replacement of Phe (F) by Tyr (Y) to allow detection at 280 nm
Modification 5 (peptide YVC)	Replacement of Ala by Val to enhance peptide lipid solubility and Phe (F) by Tyr (Y) to allow detection at 280 nm

Lysine based heptapeptides modifications

	Proposal
Original peptide (LIS)	original peptide containing Lysine
Modification 1 (peptide FVK)	Replacement of Ala by Val to enhance peptide lipid solubility
Modification 2 (peptide WAK)	Replacement of Phe (F) by Trp (W) to allow detection at 280 nm
Modification 3 (peptide WVK)	Replacement of Ala by Val to enhance peptide lipid solubility and Phe (F) by Trp (W) to allow detection at 280 nm
Modification 4 (peptide YAK)	Replacement of Phe (F) by Tyr (Y) to allow detection at 280 nm
Modification 5 (peptide YVK)	Replacement of Ala by Val to enhance peptide lipid solubility and Phe (F) by Tyr (Y) to allow detection at 280 nm

Solid phase peptide synthesis (SPPS)





- Cyclic step-wise construction of a peptide chain attached to an insoluble polymeric support
- Fmoc is the α-amino protecting group
- Carboxyl group of each incoming amino acid is activated by coupling reagents and couples with the α-amino group of the preceding amino acid.
- Fmoc is removed with 4-methylpiperidine and side-chain protectors by TFA
- The peptide chain is extended by repetition of the synthesis cycle.

- Purification: HPLC
- Degree of purity > 95%
- Characterization: Mass spectrometry

Reaction samples

Chemicals

UV filter stock solutions and positive controls (100 mM)

Substances in ACN: DMSO (1:1)

Substances 100 mM

DNCB

Vial + insert

Peptides

Cysteine and cysteine analogs

Phosphate buffer pH 7.5

Cisteína

Cysteine 0.667 mM

CYS

75 µl cysteine
20 µl ACN
5 µl chemicals
(1:10) 0.5 mM CYS:
5 mM chemicals

Lysine and lysine analogs

Ammonium acetate buffer pH 10.2

Lisina

Lysine 0.667 mM

LYS

75 µl Lysine
25 µl chemicals (1:50)
0.5 mM LYS:
25 mM chemicals

(Gerberick et al., 2004, 2007, OECD TG 442C)

Reaction and analysis

Protected from the light

24h

Centrifugation
40 min with 400 xg;
Hermle Z383K.
(only for CYS)

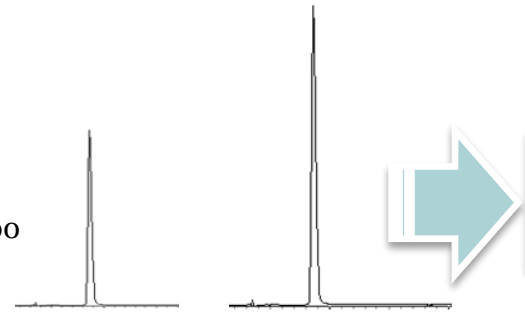
HPLC (Shimadzu)
MP: isocratic elution: 50% phase A (0.1% TFA in water) and 50% phase B (0.085% TFA in acetonitrile)

Detection at UV (220 and 280 nm)
C18 column (Agilent® C18, 100 mm, 5µm);
flow: 0.35 mL /min.

(Gerberick et al., 2004, 2007, OECD TG 442C)

Prediction models

$$Percent\ of\ peptide\ depletion = \left[1 - \left(\frac{Peptide\ peak\ area\ in\ replicate\ injection}{Mean\ peptide\ peak\ are\ in\ reference\ controls\ C}\right)\right] \text{ x } 100$$



Predictive model based on cysteine and lysine depletion (OECD, 2015).

Mean of CYS and LYS depletion (%)	Reactivity Class	Prediction
0% ≤ CYS/LYS Depletion ≤ 6.38%	None or minimal reactivity	Negative
6.38% < CYS/LYS Depletion ≤ 22.62%	Low reactivity	Positive
22.62% < CYS/LYS Depletion ≤ 42.47%	Moderate reactivity	
42.47% < CYS/LYS Depletion ≤ 100%	High reactivity	

Predictive model based only on cysteine depletion

Cysteine depletion (CYS) (%)	Reactivity Class	Prediction
0% ≤ % CYS Depletion ≤ 13.89%	None or minimal reactivity	Negative
13.89% ≤ CYS Depletion ≤ 23.09%	Low reactivity	Positive
23.09% ≤ CYS Depletion ≤ 98.24%	Moderate reactivity	
98.24% ≤ CYS Depletion ≤ 100%	High reactivity	

(Gerberick et al., 2004, 2007, OECD TG 442C)

Results and discussion

Screening - Depletion assay with Cysteine based heptapeptides

	Test chemicals (positive controls and UV filters)	Analysis in 220 nm	Analysis in 280 nm	Proficiency substances (range of % cysteine peptide depletion) (OECD TG 422C)
CYS (FAC)	DNCB	99.9 ± 0.0	-	90-100
	CINA	63.1 ± 3.5	-	60.8-100
	AVO	1.9 ± 3.3	-	-
	BP-3	7.9 ± 8.1	-	-
	OC	10.5± 9.1	-	-
YAC	DNCB	100 ± 0.0		90-100
	CINA	0.5 ± 0.9		60.8-100
		44.5.2 ± 15.1		
	AVO	20.1± 21.8		-
	BP-3	15.2 ± 12.6		-
	OC	18.7 ± 21.2		-
YVC	DNCB	77.6 + 3.8		90-100
	CINA	18.9 ± 2.0		60.8-100
		5.7 ± 6.8		
	AVO	0.0 ± 0.0		-
	BP-3	0.0 ± 0.0		-
	OC	0.0 + 0.0		-

Screening - Depletion assay with Lysine based heptapeptides

	Test chemicals (positive controls and UV filters)	Analysis in 220 nm	Analysis in 280 nm	Proficiency substances (range of % lysine peptide depletion) (OECD TG 422C)
LYS (FAK)	DNCB	20.6 ± 2.7		15 – 45%
	CINA	63.9 ± 1.2		40.2 – 69.0%
	AVO	0.0 ± 0.0		-
	BP-3	1.6 ± 1.5		-
	OC	0.0 ± 0.0		-
WVK	DNCB	13.8 + 5.8	15.2 + 6.7	15 – 45%
	CINA	56.4 ± 4.0	93.6 ± 11.1	40.2 – 69.0%
		61.9 ± 1.1	79.5 ± 3.7	
	AVO	20.4 ± 15.0	38.8 ± 20.1	-
	BP-3	0.0 ± 0.0	0.0 ± 0.0	-
	OC	21.7 ± 3.3	41.3 ± 4.6	-
YVK	DNCB	49.7± 2.6		15 – 45%
	CINA	0 ± 0		40.2 – 69.0%
		51.7 ± 6.4		
	AVO	0.0 ± 0.0		-
	BP-3	0.0 ± 0.0		-
	OC	0.0 ± 0.0		-
FVK	DNCB	37.2 ± 32.2		15 – 45%
	CINA	69.0 ± 3.7		40.2 – 69.0%
		43.2 ± 37.4		
	AVO	18.2 + 14.7		-
	BP-3	0.0 ± 0.0		-
	OC	10.0 + 17.3		-
YAK	DNCB	40.6 ± 1.8		15 – 45%
	CINA	43.7 ± 34.3		40.2 – 69.0%
		20.2 ± 34.9		
	AVO	0.0 ± 0.0		-

Proficiency of FVK analysis – equivalence to original DPRA

	FVK		
Substances	Mean	SD	Proficiency substances - range of % lysine peptide depletion (OECD TG 442C)
2,4-Dinitrochlorobenzene	25.3	5.8	15-45
Oxazolone	29.3	3.2	10-55
Formaldehyde	5.72	5.0	0-24
Benzylideneacetone	0.0	0.0	0-7
2.3-Butanedione	0.0	6.76	10-45
1-Butanol	3.0	3.5	0-5.5
Lactic Acid	3.4	3.0	0-5.5
4-Methoxyacetophenone	3.5	6.1	0-5.5
6-Methylcoumarin	0.0	0.0	0-5.5

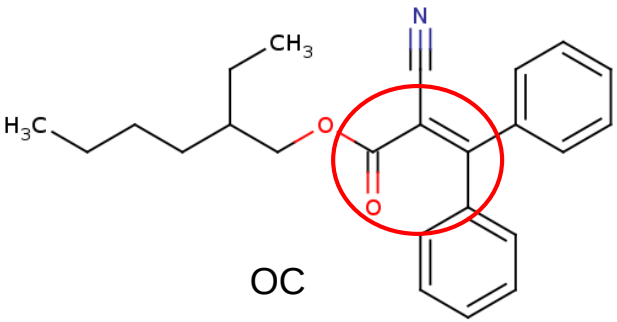
USP prediction models based on CYS and FVK or only in FVK depletion (OECD TG 442C)

Mean of CYS and FVK depletion (%)	Reactivity Class	Prediction
0% ≤ CYS/FVK or only FVK depletion ≤ 6.38%	None or minimal reactivity	Negative
6.38% < CYS/FVK or only FVK depletion ≤ 22.62%	Low reactivity	Positive
22.62% < CYS/FVK or only FVK depletion ≤ 42.47%	Moderate reactivity	
42.47% < CYS/FVK or only FVK depletion ≤ 100%	High reactivity	

substance	CYS Depletion	LYS Depletion	FVK Depletion	CYS and LYS mean	CYS and FVK mean	Reactivity based on CYS LYS mean	Reactivity based on CYS	Reactivity based on CYS FVK mean	Reactivity based on FVK	DPRA prediction model	USP prediction model
DNCB	99.9±0.0	20.6±2.7		60.3		High	High			+/+	
CINA	63.1±3.5	63.9±1.2		63.5		High	Moderate			+/+	
AVO	1.9±3.3	0.0±0.0		0.9		None or minimal	None or minimal			-/-	
BP-3	7.9±8.1	1.6±1.5		4.7		None or minimal	None or minimal			-/-	
OC	10.5±9.1	0.0±0.0		5.2		None or minimal	None or minimal			-/-	
DHHB	8.2±3.1	0.0±0.0		4.1		None or minimal	None or minimal			-/-	
OCTZ	5.3±5.8	-*		-		-	None or minimal			-	
OMC	6.9±6.7	2.0±2.0		4.5		None or minimal	None or minimal			-/-	
DNCB	99.9±0.0		37.2±32.2		68.55			High	Moderate		+/+
CINA	63.1±3.5		69.0±3.7 43.2±37.4		66.05 53.15			High High	High High		+/+ +/+
AVO	1.9±3.3		16.4±5.9		9.15			Low	Low		+/+
BP-3	7.9±8.1		0.0±0.0		3.95			None or minimal	None or minimal		-/-
OC	10.5±9.1		29.5±2.4		20.00			Low	Moderate		+/+
DHHB	8.2±3.1		6.1±5.6		7.15			Low	None or minimal		**+/-**
OCTZ	5.3±5.8		0.0±0.0		2.65			None or minimal	None or minimal		**-/-**
OMC	6.9±6.7		8.2±5.9		7.55			Low	Low		+/+

UV filters

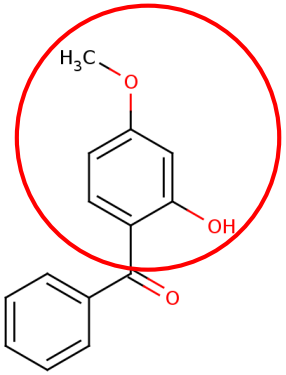
α, β-unsaturated carbonyl group: electrophile



OC

emergent photoallergen and potential contact allergic reactions

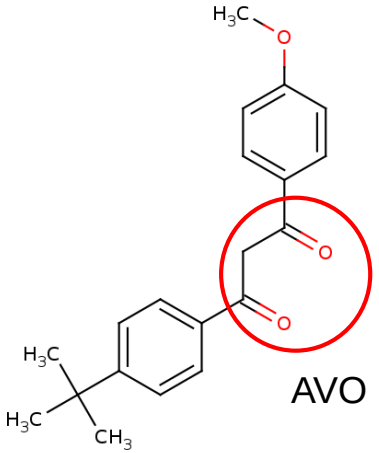
o-alkyl resorcinol precursor
Electrophile Michael addition



BP-3

photodermatitis and contact allergic

1,3-diketone, Electrophile Michael addition



AVO

contact and photo-allergic reactions both of which may lead to contact dermatitis

(Bryden et al., 2006; Nash, 2006; Sushko, 2012; Avenel-Audran et al., 2010; Delplace and Blondeel, 2006; Karlsson et al., 2011, Pigatto et al., 2008)

Photopatch studies

A 20-year analysis of previous and emerging allergens that elicit photoallergic contact dermatitis

Frank C. Victor, MD, David E. Cohen, MD, MPH, and Nicholas A. Soter, MD
New York, New York

Table IV. Summary of photopatch test studies

Location	n	Study period	Most frequent allergens
Scandinavia[5]	1993	1980-1985	Musk ambrette, PABA, promethazine, chlorpromazine
United States[6] (Minnesota)	70	1980-1985	Chlorpromazine, musk ambrette, promethazine
United Kingdom[7]	2715	1983-1998	Sunscreens, chlorpromazine, promethazine, musk ambrette
United States[4] (New York)	187	1985-1990	Sunscreens, antimicrobial agents, fragrances
Austria, Germany, Switzerland[8]	1129	1985-1990	Tiaprofenic acid, Fentichlor, carprofen, 4-isopropyl-dibenzoylmethane
United States[3] (New York)	138	1986-1993	Sunscreens, fragrances, antimicrobials agents
Netherlands[9]	44	1989-1994	Chlorpromazine, promethazine, musk ambrette
Austria, Germany, Switzerland[10]	1261	1991-1997	Fentichlor, carprofen, chlorpromazine, 2-hydroxy-4-methoxybenzophenone
Australia[11]	81	1991-1999	Oxybenzone, benzophenone-4
France[12]	2067	1991-2001	Sesquiterpene lactone, ketoprofen, benzophenone, dibenzoylmethane
India[13]	50	1994-1999	Musk ambrette, chlorpromazine, promethazine, balsam of Peru
Netherlands[9]	55	1995-1999	Eusolex 8020, Parsol 1789, benzophenone-3
United Kingdom and Europe[14]	1155	2000-2002	Benzophenone-3
United States[15]	182	2000-2005	Medications, sunscreens, fragrances, antiseptics
Italy[16]	1082	2004-2006	Ketoprofen, piroxicam, promethazine, octocrylene

(J Am Acad Dermatol, 2010)

Conclusions

- UV-filters presented positive results for the cutaneous sensitization potential, especially for avobenzone and octocrylene, which are described in the literature as low sensitizers.

- The proposed model presents different results compared to the DPRA model, since the UV-filters became more soluble and could interact better with peptide analogues

Faculdade de Ciências e Engenharia

PeSEAp

Peptídeos: Síntese, Otimização e Estudos Aplicados