

Acute dermal toxicity and sensitization studies of novel nano-enhanced UV absorbers

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Abstract: Acute dermal toxicity and sensitization studies of novel nano-enhanced UV absorbers:

Joanna PIASECKA-ZELGA, *et al.* Institute of Occupational Medicine, Research Laboratory for Medicine and Veterinary Products in the GMP Quality System, Poland—Background:

Many employees working outside are exposed to the harmful effects of UV radiation. A growing problem is also sensitization to textile materials and allergic reactions to active compounds. Groups of inorganic UV blockers with nanoparticles may provide superior properties over organic UV absorbers with relatively less potential of provoking dermatitis.

Objectives: To assess acute dermal irritation and sensitization of nano UV absorbers.

Materials & Methods: Five UV absorbers with nano-sized particles (Z11, TiO₂ – SiO₂ [TDPK], TK44, TK11, A8G) and 2 vehicles (paste-based on 10% PEG, and dispersion with 1% HEC) were tested. Acute dermal irritation was tested using group of 3 rabbits for each absorber. The sensitization study was carried out on groups of 15 guinea pigs for each tested textile with a UV absorber showing an Ultraviolet Protection Factor (UPF)>40. This research was designed according to OECD Test Guideline No. 404 and 406, and 21 rabbits and 60 guinea pigs were used in the study. **Results:** In acute dermal irritation, Z11 and A8G modifiers and the analyzed paste gave results of 0.047 to 0.33 which classifies them as barely perceptible irritants, whereas the other analyzed modifiers and dispersion gave results of 0.00 and were classified as nonirritating. Only the textile with TK 11 did

not have UPF>40. The analyzed barrier materials were classified as nonsensitizers (TDPK, A8G) or mild sensitizers (TK44, Z11). **Conclusions:** None of the analyzed materials or modifiers induced major skin reactions in animals. Therefore, they present low risk of provoking skin reactions in humans.

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Key words: Barrier textiles, Contact allergy, *In vivo*, Irritation test, Ultraviolet absorber

Exposure to UV radiation can cause a variety of symptoms and diseases, including pigmentation, telangiectasias, actinic keratoses, cheilitis actinica, photoaging, and skin cancer¹. Between two and three million people are diagnosed with non-melanoma skin cancers each year. This number has increased from 3 to 8% among white-skinned populations in Europe, Australia, Canada and United States^{2,3}. Loss of the ozone layer is further contributing to this result, and increasing the exposure of the population. Research indicates that 10% loss of the ozone layer increases the risk of basal or squamous cell carcinoma occurrence in the range of 30 to 50%^{4,5}.

Some professions have prolonged exposure to UV radiation and may be more susceptible to occupational diseases and cancers. It is noted that 5 to 10% of the adult European population work outdoors¹, and construction workers, farmers or military officers have higher risk of acquiring undesirable symptoms⁶.

Major mutations created by UV radiation are cyclobutane pyrimidine dimers and 6–4 photoproducts. Those mutations can be found in *p53* and *PTCH* tumor suppressor genes and the *ras* oncogene. What is more, UVR stimulates cell proliferation through signal transduction initiated on cellular membrane, and suppress immune responses, which can result in skin cancer development^{7,8}.

The World Health Organization recommends the

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prevention of harmful UV exposure through wearing sunglasses, using formulas with filters, and wearing loose-fitting full-length clothes⁹). However, many hours of sun exposure requires special attention and the development of solutions that provide adequate protection for workers. For this reason, attention is focusing on the introduction of special substances into fabrics, which may provide them with protective effects. Work on improving the formula of UV absorbers has been in progress for many years now. UV absorbers are substances which are capable of selectively absorbing short wavelength solar radiation and dissipating the absorbed energy back to the environment¹⁰. An effective UV absorber should be water-soluble, show good affinity to the fiber, remain stable against UVR and dissipate the absorbed energy to avoid degradation or loss in color¹¹.

One of promising group of inorganic UV blockers are nanoparticles, such as nano-TiO₂ and nano-ZnO, which increase the barrier properties of modified materials¹². Furthermore, metal oxide nanoparticles are chemically stable when exposed to high temperatures, are capable of photocatalytic oxidation and are mostly non-toxic^{13, 14}. Nanoparticles can enhance the durability of treated fabrics better than ordinary materials. That is because of their possession of large surface area and high energy which enhances their affinity for materials resulting in increased stability of the textile functions¹⁴. Especially TiO₂ and ZnO nanoparticles are widely used in cosmetics and sunscreens for UVR protection¹⁵.

Although some studies have already reported the toxicity of inorganic nanoparticles on the skin, but knowledge of the cytotoxicity and genotoxicity of such particles is still limited¹⁶. Penetration of ZnO nanoparticles through the skin, as a possible side effect of sunscreens, has been tested using white Yorkshire pigs with sunburned skin. It was found that ZnO nanoparticles penetrate just one or two layers of the stratum corneum¹⁷. A study of Sprague-Dawley rats showed collagen loss after dermal application of nano zinc oxide¹⁸. These results may indicate that, despite the beneficial absorption of harmful radiation, there are possible adverse effects of ZnO nanoparticles after prolonged contact with the skin which need to be further investigated. One of the possible reasons for the observed toxicity may be the production of reactive oxygen species (ROS) by nanoparticles leading to oxidative stress^{19, 20}.

Despite their many good qualities and properties, the wide use of nanoparticles in filters and clothes causes greater skin exposure, which may increase adverse effects through the potential risk of percutaneous absorption and ROS-mediated skin aging²¹. In addition, an increasing number of subjects are

exhibiting symptoms of textile allergic contact dermatitis. Allergic reactions can be triggered by specific dyes or fiber types²². That is why modifications of the manufacturing process or the physical properties of nanoparticles are tested in order to diminish their harmful effects on organism, while maintaining high UV absorption properties. Potential side effects may also arise from the dispersions or vehicles used to stabilize nanoparticles in textile structures. For example, polyethylene glycol (PEG) is considered to be a slight to moderate irritant and therefore should not be used on damaged skin because it may cause systemic toxicity and contact dermatitis in burn patients²³. Materials covered with UV absorbers should only have positive and protective effects on outdoor workers and exposed people. We must therefore eliminate skin symptoms and allergic reactions, and this was the main focus of our study.

Materials and Methods

Animals

Twenty-one Imp:BN New Zealand white rabbits, outbred, both sexes, approximately 3 months old, body mass 3,000–4,000 g, were used in the experiments to evaluate of irritation effects. This species is suitable for predicting human skin irritants²⁴. Sixty Imp:D-H Dunkin-Hartley guinea pigs, outbred, both sexes, aged approximately 2–3 months, body mass 350–500 g, were used in this study to evaluate sensitization. Both the Imp:BN New Zealand white rabbits and the Imp:D-H guinea pigs were provided by the Institute of the Occupational Medicine (own breeding). The animals were housed in stainless steel cages, containing sterile paddy husk as bedding, in ventilated animal rooms. They were acclimated in a controlled environment (temperature: 20 ± 2°C; humidity: 50 ± 10%; automatically regulated lighting time 12h: 12h) with *ad libitum* access to water and a commercial laboratory food. All studies involving laboratory animals were carried out with the prior consent of the Local Ethics Committee No. 9 for animal experiments of Institute of Occupational Medicine in Lodz, Resolution No. 2/ŁB 503/2010 on 22 March 2010.

Reagents

Freund's adjuvant, sodium dodecyl sulfate and cottonseed oil used for the sensitization tests were supplied by Sigma-Aldrich Corporation (Poznan, Poland), while saline solution (0.9% NaCl) was provided by Baxter Manufacturing Sp. z o.o. (Lublin, Poland).

85% α -Hexyl cinnamaldehyde used for the validation of the skin sensitization method was supplied by Sigma-Aldrich Corporation (Poznan, Poland). Aqua pro injection provided by Baxter Manufacturing

Sp. z o.o. (Lublin, Poland), and the sterile gauze compresses, 17-filiform supplied by TZMO SA (Torun, Poland), were used for the sensitization and irritation tests.

Tested modifiers and barrier materials

In the present work, we evaluated inorganic absorbers based on micronised particles of titanium dioxide, and zinc oxide and the triazine-based organic absorber, A8G, which are newly developed products which have barrier properties against UV radiation across the whole UV range. These modifiers are subject to patent EP 2565187 *New reactive triazine derivatives as ultraviolet absorbers increasing barrier properties of cellulose fibres*, and patent application PCT/PL2011/000120 *Process of manufacturing textile barrier materials*. These products were used in application tests for the modification of textiles made of cellulose and polyester fibres. The method of incorporation into the textile structure was also developed:

- inorganic absorbers: incorporation methods of dip-coating and coating
- organic absorber: exhaustion method during the dyeing process.

In order to impart protective properties against UV radiation to selected textile fabrics the textile barrier materials can be applied as elements of the protective apparel.

Hence for the evaluation of local irritation effects (rabbit) four new inorganic absorbers and one organic absorber were selected including:

- TiO₂-SiO₂ labeled modifier (TDPK) obtained from the Institute of Chemical Technology and Engineering, Poznan University of Technology, Poland^{25, 26};
- TK44 modifier – preparation of TiO₂ anatase variety – A11 (Police S.A.) surface-modified N-2-(aminoethyl)-3-aminopropyl trimethoxysilane in the ratio of 1:100, obtained from the Institute of Chemical Technology and Engineering, Poznan University of Technology, Poland²⁷;
- Z11 modifier – zinc oxide, containing nanometer sized particles (average particle diameter 396 nm, polydispersion 0.161), obtained from the Institute of Chemical Technology and Engineering, Poznan University of Technology, Poland²⁸;
- TK11 modifier – TiO₂ preparation rutile R211 variant (Police S.A.) surface-modified 3-methacryloxypropyltrimethoxysilane 1:100, obtained from the Institute of Chemical Technology and Engineering, Poznan University of Technology, Poland;
- Reactive UV absorber type A8G obtained from the Institute of Polymer and Dye Technology, Lodz University of Technology.

Additionally the paste and dispersion alone (i.e. without absorbers) were tested in order to assess their potential role in provoking local irritation:

- For the coating method, the paste consisting of 10% PEG (polyethylene glycol), 10% Revacryl 247 (styrene/acrylic ester copolymer dispersion of low viscosity), 3% Lutexal TX 4733 (acrylic thickener) and 77% water was provided by the Textile Research Institute of Lodz, Poland;
- For the dip-coating method, the dispersion without absorber containing 10% PEG (polyethylene glycol), 1% HEC (hydroxyethylcellulose) and 89% water was provided by the Textile Research Institute of Lodz, Poland;

The analysed inorganic and organic absorbers were applied to the following:

- textile materials (polyester woven and nonwoven fabrics and woven fabric made of cotton fibres) containing in their structures selected inorganic absorbers (TK11 modifier, TK44 modifier, TDPK modifier and Z11 modifier) based on micronized particles of titanium dioxide and zinc oxide, which provide the best protective properties against UV radiation. Applied absorbers called *physical blockers* reflect and absorb UV radiation. The newly developed inorganic absorbers were subjected to surface modification to develop/increase their specific surface and to improve their adhesive properties to fibres.
- knitted fabric made of cotton fibres modified with organic absorber A8G. Application of the reactive-type absorber is advantageous for the modification of fabrics made of cellulose fibres due to groups in the absorber structure which are capable of reacting with cellulose hydroxyl groups. Chemical bonding of such an absorber with cellulose fibre provides durable barrier properties against UV radiation.

All 4 textiles and the knitted fabric underwent UPF (Ultraviolet Protection Factor) testing, according to UPF Testing Protocol EN 13758-1 in conjunction with EN 13758-2, which are standard requirements for the testing and labelling of fabrics to be called of UV-protective²⁹). UPF testing can also be performed by American Association of Textile Chemists and Colorists (AATCC) Method 183 or the Australian Standardization Institute (AS/NZS 4399). Rates between 15–24 indicate good performances, blocking up to 95.9% of the UV radiation falling on the fabric. Textiles rated between 25–39, reveal very good blocking capacity (of up to 97.4%), whereas fabrics rated 40–50+ demonstrate excellent UV protective ability, being able to block of more than 97.5% of the UV

radiation falling on the fabric. In EN standards only textiles that have ratings of at least 40 in the test are labelled sun-protective. In this study, the textile material with the TK 11 modifier did not have a rating greater than 40 in UPF testing (it was 35) and it was excluded from further test. The remainder of the analysed materials which had ratings greater than 40 in the UPF test were included in the sensitization study.

Thus, the following 4 textile barrier materials with selected UV filters dispersions were evaluated for their sensitization properties:

- Polyester fibers (Lentex S.A., Lubliniec, Poland) with paste containing 3% by weight $\text{TiO}_2\text{-SiO}_2$ (TDPK) modifier,
- Polyester fabric (Tkanfarb Sp. z o. o., Lodz, Poland) with dispersion containing 3% by weight TK44 modifier,
- Noris cotton fabric (YORK Warehouse, Radom, Poland) with dispersion containing 3% by weight Z11 modifier,
- Cotton knitwear (AGW - Kolor Sp. z o. o., Lodz, Poland) with reactive UV absorber type A8G in intensity 0.5% in relation to dry textile end product weight.

Research method

1) Acute Dermal Irritation

The research was carried out according to the OECD Test Guideline (TG) 404 - Acute skin irritation/caustic effect, B.4 Procedure – Acute toxicity (Acute Dermal Irritation/Corrosion).

Acute Dermal Irritation studies were performed on 3 Imp:BN New Zealand albino rabbits for each tested substance. The total number of rabbits used in this test was 21. The test was designed to assess the irritation effect on the skin of the various new UV filter dispersions intended for application on textiles.

The day before application of the dispersions, the investigated rabbits, fur was removed from the dorsal area on both sides of the trunk. On the day of investigation, UV filter dispersion samples of 0.5 g were applied to sterile gauze and then applied to the skin on the right site of the rabbits. Similarly, on the skin on the left site of the rabbits, sterile gauze with 0.5 ml aqua pro injection was applied according to the scheme (Fig. 1). The sites treated with the reagents were protected by a non-irritating plaster. After single 4-hour exposures the plasters were removed and both the treated and control sites were washed with water. One hour later, the first reading of the treated and controlled sites was conducted. Researchers observed animals skin to determine whether erythema, scab or swelling had appeared. Subsequently, the procedure was conducted and readings were recorded every

day for two weeks from the end of the single closed exposure. The assessment of the skin irritation effect was performed on the basis of the classification of the skin reaction³⁰. Skin reactions were assessed independently for erythema and edema, based on a 0–4 grading scale. For erythema, 0 means no erythema, 1 very slight erythema/barely perceptible, 2 well-defined erythema, 3 moderate to severe erythema, and 4 severe erythema to slight eschar formation (injuries in depth). For edema, 0 means no edema, 1 very slight edema/barely perceptible, 2 slight edema (edges of area well defined by rising), 3 moderate edema (raised approximately 1 mm), and 4 severe edema (raised more than 1 mm and extending beyond the area of exposure). For each tested rabbit the Primary Irritation Score was calculated by summing the scores for erythema and edema at the test site minus the sum of the erythema and edema at the control site for the whole analysed period divided by the number of observations (i.e. 14). The primary irritation index (PII) was calculated as the arithmetical mean of the sum of the Primary Irritation Scores for all animals in the group (i.e. 3). According to OECD test guideline number 404, absorbers with a PII of 0 can be classified as non-irritant, 0.04 to 0.99 as irritation barely perceptible, 1.00 to 1.99 as slightly irritant, 2.00 to 2.99 as mildly irritant, 3.00 to 5.99 as moderately irritant, and 6.00 to 8.00 as severely irritant.

2) Guinea Pig Maximization Test (GPMT)

The guinea pig maximization test was conducted according to the methods of Magnusson and

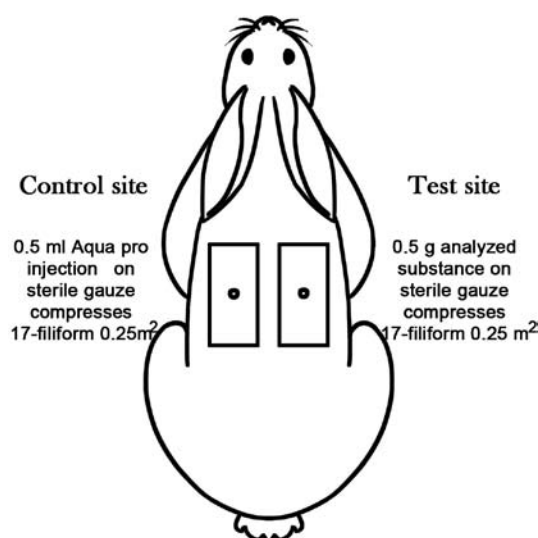


Fig. 1. Location of skin application sites.

Kligman^{30,31}) and based on the OECD Test Guideline (TG) 406 directions–Skin sensitization, B.6 Procedure – Acute toxicity (Acute Dermal Irritation/Corrosion).

The sensitization study was performed using 15 Imp:D-H guinea pigs for each material tested, a total number of 60. For each material, ten animals formed the test group and five the control group. On the day before the investigation, the guinea pigs` fur was removed from the dorsal area on the both sides of the trunk, not to injure the skin. Then, on the first day of the induction period, three pairs of intradermal injections (0.1 ml/site) in the scapular area were made (Fig. 2). Induction was also carried out in the control group, but in those animals, water was used in place of the extract sample.

The extracts were prepared in a vehicle, aqua pro injection (series number: 1306255, expiration date: 05.2016, Manufacturer: Baxter Manufacturing Sp. z o. o.). Under aseptic conditions in a laminar flow chamber, fabric was removed from the packaging unit. Textile material of a total area of 120 cm² was selected using sterile forceps and then, cut with scissors, into pieces of approximately 1 cm length. Fragments

of the textile material were immersed in 20 ml of aqua pro injection in sterile 50 ml Falcon dishes, so that the mass ratio of the sample to the volume of the extracting agent was 6 cm² per 1 ml of the sample, following ISO 10993-12:2012. After 24 hours incubation, pH and concentration were measured. On the 8th day of the experiment 0.5 g of 10% sodium lauryl sulfate in vaseline was applied on the right site of all the guinea pigs to provoke local inflammation. Then, after 24 hours, the right sites of guinea pigs were washed. Next, a sterile gauze compress with 0.5 ml sample water extract was applied to the exposed skin, stabilized with a bandage, and left for 48 hours. On day 22 of the study, a sensitization reaction was induced in all the guinea pigs. On the shaved right site of guinea pigs, a sterile gauze compress with 0.5 ml sample water extract was applied, and on the shaved left site, a sterile gauze compress with 0.5 ml aqua pro injection was applied. The sites were checked after 24 hours, and the sensitization effect was evaluated at 48, 72 and 96 hours after the sample application, using the Magnusson-Kligman Classification to evaluate the skin reactions³⁰.

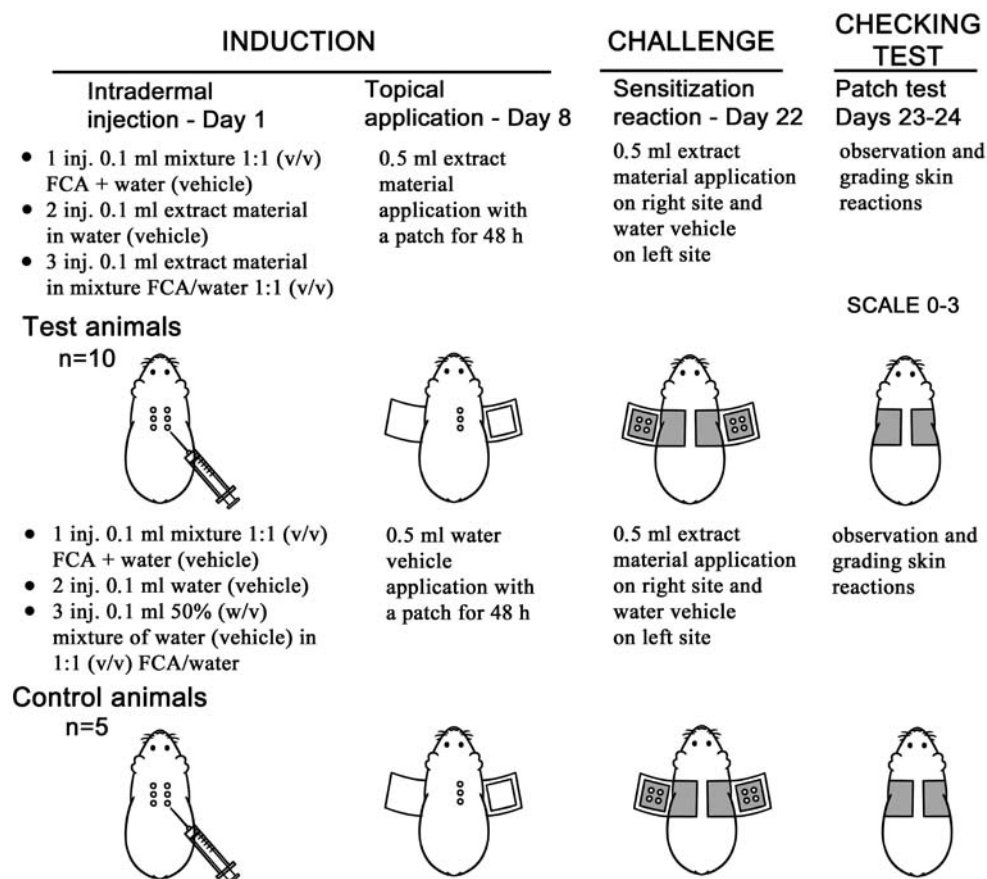


Fig. 2. Schematic illustration of the Guinea Pig Maximization Test (GPMT).

Statistical analysis

The chi-square test with Fischer's exact test was used to determine if the differences in scores between the tested and control sites or animal (depending on test) were significant. The differences in responses of the test site/group versus the control site/group were compared using the Mann-Whitney U test. Differences were considered significant for $p < 0.05$. Statistical analysis of the results was performed with the STATISTICA software for Windows (v.10 StatSoft, Inc, Tulsa OK, USA).

Results

Skin irritation test

The results of the acute dermal irritation studies are shown in Table 1. No edema reactions were observed at the tested or control sites of any analyzed rabbit. All observed skin reactions were classified according to Draize's scale as 1 (very slight erythema). No higher grades for erythema were observed. For tested UV absorbers the PII value was 0.00 except for:

- paste containing 10% polyethylene glycol, 10% Revacryl 247, 3% Lutexal TX 4733-0.047,
- type A8G absorber-0.047
- Z11 modifier-0.33.

According to the OECD classification, these three absorbers are classified as barely perceptible irritant, whereas the rest of analysed absorbers can be classified as non-irritants.

Only the Z11 modifier showed a significant difference between the scores of the control and test sites ($p = 0.0008$), as well as in the responses observed ($p < 0.001$). For both the paste and A8G modifier there were a differences between the scores of the tested sites ($p = 0.222$ for both) and in the responses observed ($p = 0.1823$ and $p = 0.1648$ respectively), but they were not statistically significant.

Sensitization test

The present study revealed that neither the polyester fibers covered with paste containing 3% by weight $\text{TiO}_2\text{-SiO}_2$ (TDPK) modifier nor the cotton fabric with reactive UV absorber type A8G of intensity 0.5% caused erythema or swelling in any guinea pig of the treated group during 96 hours of observation (Table 2, Fig. 4). Therefore, these materials can be classified as nonsensitizers.

In the case of polyester fabric with a dispersion containing 3% by weight TK44 modifier, slight erythema was observed in one guinea pig. After 72 hours this effect had disappeared. In the case of the cotton fabric coated with the dispersion containing 3% of Z11, slight erythema was observed in one guinea pig, but it had disappeared after 96 hours. In the other 9 guinea pigs of the both test groups (TK44,

Z11), no skin allergic reaction was observed at the treated sites. There were no visual changes in skin reactions in the control groups. These materials can be classified according to the Magnusson and Kligman Classification for evaluation of skin reaction²³⁾ as mild sensitizers.

There were no significant differences between the scores of the control and test groups as well as in the responses observed in the textiles provoking allergic reactions (TK44, Z11).

Discussion

Preclinical evaluations of irritation and sensitization of the new generation barrier materials are necessary before clinical trials on humans may even be considered. The results of the present research provide interesting information about possible interactions of UV barrier materials enhanced with nanoparticles through *in vivo* toxicity testing. The effects we were able to determine include: induction of inflammatory reaction, acute irritation reaction, and acute skin sensitization. The results of this study provide qualitative and quantitative data, for the careful evaluation of the allergenicity of UV filter dispersions and textile materials (slight, medium or significant sensitivity potential on the basis of guinea pigs' sensitization frequency in the treated group).

The *in vivo* test is the method of choice for determining the irritation potential of chemicals²⁹⁾. Since we tested new formulas of UV filters dispersions, no information was available in the literature regarding our particular mixtures in relation to their Primary Irritation Index. However, some of the components of the investigated formulas have already been tested for their toxicological effects.

The Primary Irritation Index of each analyzed UV filter dispersions was within the range of 0.00 to 0.33. The materials did not provoke to significant allergic reactions. According to ISO 10993-10:2011, substances with a PII below 0.5 are safe for animals and humans. Therefore, we can conclude that the UV filter dispersions examined by us can be considered as safe products. However, we cannot ignore the fact that workers exposed to UVR will be in continuous contact with fabrics saturated with these filter dispersions, which may provoke potential allergies when used for a prolonged time. Therefore, further studies are warranted. In the case of Z11 modifier (nanometer sized particles of zinc oxide), two out of three tested rabbits showed skin reactions after a single exposure, and this effect could multiply with repeated exposures. Standard error evaluation (Fig. 3) indicates that some animals may have magnified reaction to this filter.

In the available literature, there are few studies of the toxicity of ZnO nanoparticles, which are included

Table 1. Acute dermal irritation test: Erythema and edema scores with the Primary Irritation Index of the tested UV absorbers

Absorbers	Rabbit	Days																												PIS	PII
		1		2		3		4		5		6		7		8		9		10		11		12		13		14			
		T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C		
TiO ₂ – SiO ₂ (TDPK)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0	0,00
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0	
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0	
TK44	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0	0,00	
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0		
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0		
TK11	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0	0,00	
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0		
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0		
Z11	1	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0,78	0,33	
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0			
	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0,21		
A8G	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0	0,047		
	2	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,14			
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0			
Paste*	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0	0,047		
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0			
	3	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,14			
Dispersion"	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0	0,00		
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0			
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0			

t-tested site of rabbit C- control site of rabbit.

* Paste consisting of 10% PEG (polyethylene glycol), 10% Revacryl 247 (styrene/acrylic ester copolymer dispersion of low viscosity), 3% Lutexal TX 4733 (acrylic thickener) and 77% water.

" - Dispersion without absorber containing 10% PEG (polyethylene glycol), 1% HEC (hydroxyethylcellulose) and 89% water.

Primary Irritation Score (PIS)=sum of the scores for erythema and edema at the test site minus the sum of scores for erythema and edema at the control site for a particular rabbit in the whole analysis period divided by number of observations, e.g. 2/14=0,14.

Primary Irritations Index (PII)= $PIS_{rabbit1} + PIS_{rabbit2} + PIS_{rabbit3}$ divided by number of tested animals e.g. (0,78 + 0,0 + 0,21)/3= 0,33.

in the Z11 modifier. Oral administrations of these particulates to healthy adult mice caused alarming changes in their livers, kidneys and lungs, and their liver enzymes (ALT, AST) were raised³². However, the possibility of skin penetration is a major concern when discussing the toxicity of ZnO^{34, 35}. Although some papers state that a particle diameter of less than 5 nm enables skin penetration, it was demonstrated that deep penetration is only possible in cases of impaired skin³⁶. It should be noted, that

the Z11 modifier did not present an acute toxicity, however more tests should be performed to examine chronic exposures, especially in light of evidence that UV protection materials are used to protect already damaged skin.

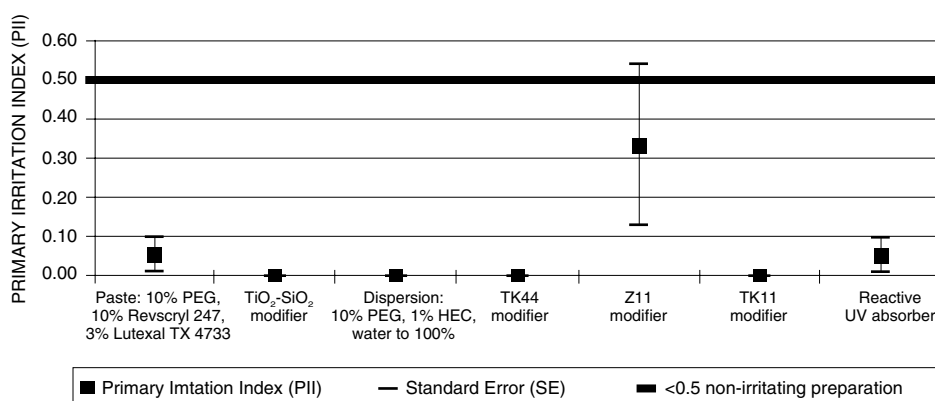
The remaining filter dispersions showed no reaction or minimal irritation effects, which in terms of observation and statistical analysis may indicate their safety.

In the Guinea Pigs Maximization Test (GPMT) of

Table 2. Results of textile materials` sensitization reaction tests

Analyzed textile material	Number of animals		Number of animals with lesions			Type of lesions	Grade of sensitization
	Test	Control	48 h	72 h	96 h		
Polyester fibers covered with paste containing 3% by weight TiO ₂ -SiO ₂ (TDPK) modifier	10	5	0/10 0/5	0/10 0/5	0/10 0/5	lack of visible lesions	0
Polyester fabric with dispersion containing 3% by weight TK44 modifier	10	5	1/10 0/5	0/10 0/5	0/10 0/5	1-Discrete erythema	II
Noris cotton fabric with dispersion containing 3% by weight Z11 modifier	10	5	1/10 0/5	1/10 0/5	0/10 0/5	1-Discrete erythema	II
Cotton knitwear with reactive UV absorber type A8G in intensity 0.5% in relation to dry textile end product weight	10	5	0/10 0/5	0/10 0/5	0/10 0/5	lack of visible lesions	0

Grades of sensitization based on percent of animals response in a group: 0%–Grade 0–nonsensitizer; >0–8%–Grade I–weak sensitizer; 9–28%–Grade II–mild sensitizer; 29–64%–Grade III–moderate sensitizer; 65–80%–Grade IV–strong sensitizer; >80%–Grade V–extreme sensitizer.

**Fig. 3.** Primary Irritation Index (PII) and Standard Error (SE) of the irritation tests results.

Magnusson-Kligman, all skin reactions are observed and recorded, including systemic reaction resulting from induction and provocation procedures.

The polyester fibers with paste containing 3% by weight TiO₂-SiO₂ (TDPK) modifier, and cotton fabric with reactive UV absorber type A8G of intensity 0.5% did not cause an allergic reaction in any guinea pig. Polyester fabric with dispersions containing 3% by weight TK44 modifier and cotton fabric with dispersion containing 3% by weight Z11 caused an allergic reaction in one of the guinea pigs, which is 10% of the number of animals tested (Fig. 4). According to the Globally Harmonized System of Classification and Labelling of Chemicals, substances, which in

the GPMT of Magnusson-Kligman cause sensitization reactions in less than 30% of the animals, should cause sensitization reactions in humans and should not be classified as substances with sensitization properties. A reaction in one out of ten guinea pigs is negligible, and it should not be assumed, that substances with this kind of results are excessively dangerous. Therefore, it might be considered that the textile materials we investigated present minor risk of serious allergic reactions in humans.

We can assume that the analyzed UV filter dispersions and textile materials will not cause serious, permanent damage, inflammation or lesions in the outer epidermis layer, as observed in cases of signifi-

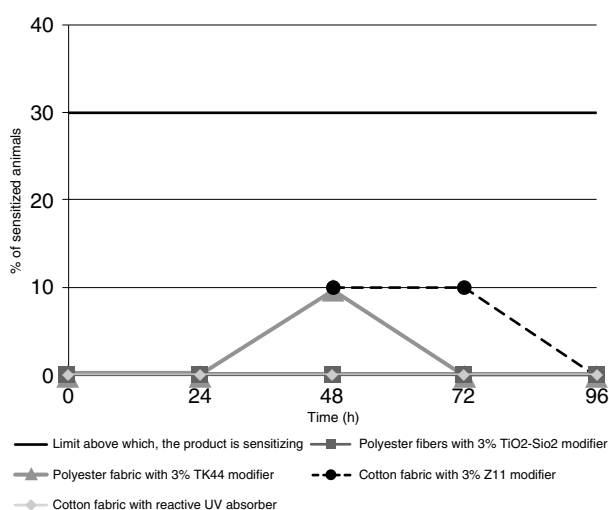


Fig. 4. Textile materials sensitization effects in the Guinea Pig Maximization Test (GPMT).

cant skin irritation. The results of our tests of the dispersion filters and impregnated materials show an insignificant toxic activity. However, more research on the components used in filters, the dose applied to the materials and the length of skin contact, should be performed.

Conclusion

The modifiers evaluated for irritation effects had obtained Primary Irritation Indexes ranging from 0.00 to 0.33, which categorize them as nonirritating or barely perceptible irritating modifiers. Sensitization effects of analyzed barrier materials were visible in fewer than 10% of animals, which categorize them as non-sensitizing or mild sensitizing materials.

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