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# Safety and tolerability of two emollients containing complex targeting *Staphylococcus aureus* biofilm formation

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## Introduction

**Increased skin colonization by *Staphylococcus aureus* is associated with atopic eczema (AE) severity. Reduction of *S. aureus* levels on the skin results in an improvement in the clinical condition. Therefore, the aim of this study was to evaluate the reduction od S. aureus biofilm formation, with little impact on *Staphylococcus epidermidis* biofilm, of two emollients for face and body care (no. 16923 bath and shower emulsion and no. 16926 special body and face formulation) containing complex of two ingredients (oligofructants from *Ophiopogon japonicus* and acetyl heptapeptide-4). Moreover safety and tolerability of latter products were estimated.**

## Materials and Methods

**In plates, bacterial suspensions of strains *Staphylococcus aureus* MSSA (83254), MRSA (BH1CC), ATCC 6538 and three strains of *S. epidermidis* (RP62A, 1457 and 12228) were mixed and incubated with several dilutions of tested emollients for 24 hrs, stained with crystal violet.**

**Also MTT cytotoxicity *in vitro* (L929 cells) and irritation potential *ex vivo* on EpiDerm skin model were measured according to ISO 10993.**

**In addition to this, the severity of AE and occurence of flares-up in children was tested in a preliminary studies in comparison to medical treatement.**

## Results – *in vitro* cytotoxicity and *ex vivo* irritation potential

**The tested products did not show skin irritation potential (mean tissue viability – above 90%). It has been also confirmed that it was deemed as not cytotoxic (viability >70% of the control) towards L-929 cells at the concentration of at least or equal to 0,01% (16926) and 0,001% (16923).**

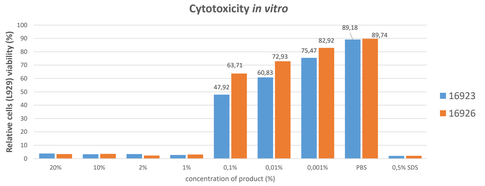


**Figure 5.** Cytotoxicity of 16923 and 16926 on L929 cells. Viability <70% of the control – cytotoxic potential. Ref – 0,5% SDS. **The tested products were non-cytotoxic** at the concentration of at least or equal to 0,001% and 0,01% respectively (cells viability: 75,47% and 72,93%).

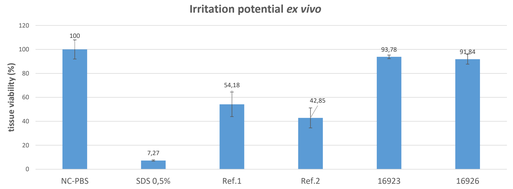


**Figure 6.** Skin irritation potential of tested on EpiDerm model. Ref 1- naphthalene acetic acid (CAS86-87-3) – non classified (non irritant). Ref 2 - cyclamen aldehyde (CAS 103-95-7) – classified (irritant, Cat. 2). Tissue viability ≤ 50% of the control (PBS) — irritant (R38). Tissue viability ≥ 50% of the control — non-irritant. **Both products are non-irritating.**

## Results – adhesion and biofilm formation

| Reduction of biofilm after exposition to tested products, as % reduction of absorbance $A_{595}$ | Reduction $A_{595}$ (%) ± SD | |
|---|---|---|
| | *S.aureus* 6538 | *S.epidermidis* 12228 |
| Product no. 16923 | 83,2 ± 2,5 | 68,9 ± 4,3 |
| Product no. 16926 | 69,8 ± 10,7 | - 174,96 ± 73,27 (Stymulation of biofilm formation) |

**Table 1.** Reduction of biofilm formation of *S. aureus* and *S. epidermidis* growing in medium containing several concentrations of tested products.

## Conclusions

- **Both products were biocompatible with the skin and well-tolerated.**
- **The influence of leave-on product 16926 on biofilm formation was dependent from bacterial strain used in tests.**
- **Rinse-off product 16923 was reducing bacteria adhesion and biofilm formation in most of concentrations used.**
- **Both products may be considered as an effective Emollient Plus type of devices to reduce flares-up in patients suffering for atopic eczema.**
- **Both products can support medical treatment or be used as first choice therapy in AE.**

## Results – dose-dependent effect on *Staphylococcus epidermidis* adhesion and formation of biofilm *in vitro*

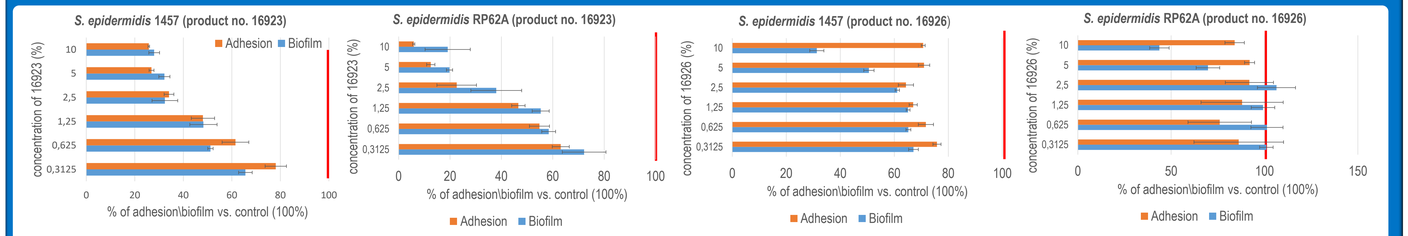


**Figure 1**. Biomas level in % due to adhesion or biofilm formation by *S. epidermidis* strains 1457 and RP62A growing in medium containing different concentrations of bath and shower emulsion no 16923. **Product reduces adhesion of *S. epidermidis* to abiotic surfaces and reduces formation of biofilm.** Result is directly propotional to concentration of product used in tests.

**Figure 2**. Biomas level in % due to adhesion or biofilm formation by *S. epidermidis* strains 1457 and RP62A growing in medium containing different concentrations of special body and face formulation no. 16926. **Product slightly reduces formation of biofilm of strain 1457**. Result is directly propotional to concentration of product used in tests up to 2,5%. **Product did not reduce biofilm formation of RP62A strain in concentrations 0,3125-2,5% but inhibits in conc. 5-10%**.

## Results – dose-dependent effect on *Staphylococcus aureus* adhesion and formation of biofilm *in vitro*

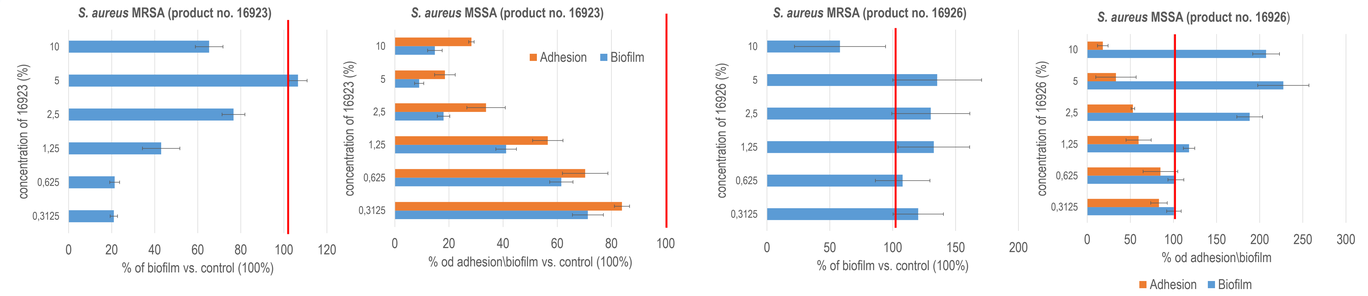


**Figure 3**. Biomas level in % due to adhesion or biofilm formation by *S. aureus* strains MRSA and MSSA growing in medium containing different concentrations of bath and shower emulsion no 16923. **For MSSA strain inhibition of growth is directly propotional to concentration of product used in tests, wheres for MRSA strain the growth is inhibited in concentrations 0,3125-2,5 % and 10%.**

**Figure 4**. Biomas level in % due to adhesion or biofilm formation by *S. aureus* strains MRSA and MSSA growing in medium containing different concentrations of special body and face formulation no. 16926. **Product is reducing adhesion of MSSA strain and increses capability of biofilm formation in concentrations 2,5-10%. Product is inhibiting biofilm formation of MRSA strain in 10% concentration.**

## Results – clinical evaluation of skin condition for product no. 16926

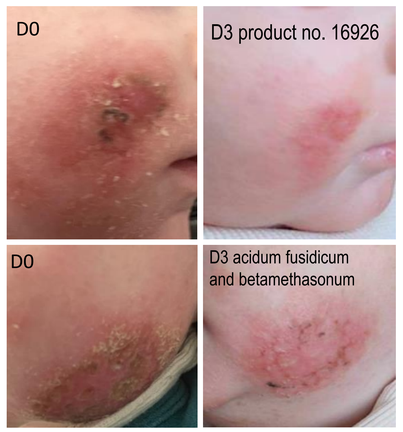


**Figure 8**. Patient ID 1A (male, 7 months). Visible flare-ups on both cheek area. Right cheek was treated with product no. 16926, and left cheek with drug acidum fusidicum and betamethasonum. **The efficacy of product no. 16926 was comparable to medical treatement.**

| Skin symptoms | Clinical evaluation of skin condition in analogue scale (n=4) | | |
|---|---|---|---|
| | DO | D7 | improvement in % |
| skin redness | 2,75 | 1,25 | 54,5 |
| edema/papules | 2,25 | 1,25 | 44,4 |
| oozing/crust | 2,25 | 0,75 | 66,7 |
| excoration | 1,75 | 1 | 42,9 |
| lichenification | 2 | 1 | 50 |
| itch (subjective) | 4,25 | 2,25 | 47,1 |
| sleep disturbance (subjective) | 3,25 | 2 | 38,5 |

**Table 2**. Clinical evaluation of skin condition in 3-point analogue scale (3-severe symptoms, 0-lack of symptoms) and subjective opinion about itch and sleep disturbane in 10-point analogue scale (10-severe, 0-lack of symptoms) of product no. 16926. **The improvement in all evaluated sympthmoms was observed**.

| Patient ID | Area of sample collection | Result | |
|---|---|---|---|
| | | D0 | D7 |
| 3A | antecubital crease | (+) *S.aureus* MSSA | negative |
| 1A | cheek | (+) *S.aureus* MSSA | negative |
| 8A | antecubital crease | (+) *S.aureus* MSSA | negative |
| 7A | cheek | (+) *S.aureus* MSSA | negative |

**Table 3**. Swabs collected from patients before and after test of product no. 16926. Before test all patients had present *S.aureus* in tested lesions. **After usage of product no. 16926, tests for presence of *S.aureus* were negative in all cases.**