

Barrier damaging effects of *n*-propanol in occlusion-modified tandem repeated irritation test: Modulation by exposure factors and atopic skin disease

Irena Angelova-Fischer^{1,2} | Maryam Soltanipoor³ | Tasja Stilla¹ |
Tobias W. Fischer^{1,2} | Sanja Kezic³ | Ivone Jakasa⁴ 

¹Department of Dermatology, University of Lübeck, Lübeck, Germany

²Department of Dermatology, Kepler University Hospital, Linz, Austria

³Coronel Institute of Occupational Health, Amsterdam UMC, Amsterdam Public Health Research Institute, University of Amsterdam, Amsterdam, Netherlands

⁴Laboratory for Analytical Chemistry, Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

Correspondence

Dr. Irena Angelova-Fischer, Department of Dermatology, Kepler University Hospital, Krankenhausstraße 9, 4020 Linz, Austria.
Email: irena.angelova-fischer@kepleruniklinikum.at

Dr. Ivone Jakasa, Laboratory for Analytical Chemistry, Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia.
Email: ijakasa@pbf.hr

Abstract

Background: Recent studies provide evidence for significant and previously underestimated barrier damaging effects of repeated exposure to 60% *n*-propanol in healthy skin *in vivo*.

Objectives: To investigate further the cumulative effects of a range of *n*-propanol concentrations relevant at the workplace in healthy and atopic dermatitis (AD) individuals, and study the modulation of the outcomes by co-exposure and host-related factors.

Methods: Healthy adult and AD volunteers were exposed to *n*-propanol concentrations from 30% to 75% in occlusion-modified tandem repeated irritation test with measurements of erythema, transepidermal water loss, capacitance, and the natural moisturizing factor (NMF) levels at baseline and after 96 hours.

Results: *n*-Propanol exerted significant barrier damaging effects even at the lowest concentration in both groups. Exposure to all *n*-propanol concentrations significantly reduced the NMF levels. Preceding low-grade trauma by occlusion/water exposure reduced the skin irritation threshold in both groups. The differences in the severity of the barrier function impairment after exposure to the same concentrations under the same conditions between the AD and control groups were significant.

Conclusions: The negative effects of cumulative exposure to *n*-propanol in healthy and atopic skin shown in the study suggest the need for critical re-evaluation of its irritant properties *in vivo*.

KEYWORDS

alcohol-based hand disinfectants, atopic dermatitis, irritant contact dermatitis, natural moisturizing factors, skin barrier

1 | INTRODUCTION

Alcohol-based hand disinfection, repeated washing, and occlusion are important extrinsic factors for development of cumulative irritant

hand eczema (HE) and contribute significantly to the sum of events leading to clinically manifest work-related HE in professions with relevant exposure. *n*-Propanol (1-propanol) is a short-chain alcohol and a common constituent of workplace or household hand disinfectants. Similar to isopropanol (2-propanol) or ethanol, *n*-propanol exerts antimicrobial effects at a broad concentration range from 15% up to 85%.

Maryam Soltanipoor and Tasja Stilla contributed equally to this study.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2019 The Authors. Contact Dermatitis published by John Wiley & Sons Ltd.

To increase the antimicrobial activity and consequently, efficacy, in commercial preparations *n*-propanol is frequently combined with isopropanol (2-propanol) or ethanol and 30% or 45% concentrations of the irritant are found in some of the most common marketed formulations recommended for hand disinfection in hospital settings.^{1,2} Compared with alkaline soaps and detergents, short-chain alcohols are considered to have a relatively low skin irritation potential.³ However, in two recent studies, we found considerable and previously unknown negative effects of 60% *n*-propanol on the permeability barrier function and corneocyte surface topography in the healthy human skin *in vivo*.^{4,5} Therefore, in the present study, we aimed to extend our previous findings by investigating the effects of the applied irritant concentration and the relative contribution of workplace-relevant co-exposures in healthy volunteers and individuals with atopic dermatitis (AD), known to have an increased risk for irritant HE. To the best of our knowledge, the cumulative effects of *n*-propanol or other short-chain alcohols on the barrier function and properties in atopic individuals have not been investigated under controlled experimental conditions so far.

2 | METHODS

2.1 | Study population

Twenty healthy adult volunteers without a history of skin or systemic diseases (16 female and 4 male; mean age 26.2 years) and 20 individuals with AD, according to the UK working Party Criteria (17 female and 3 male; mean age 25.3 years), were included in the study.⁶ To be eligible for participation, the AD individuals had to be in a stage of clinical remission, defined as the absence of active eczema lesions within the test area or any other body site for at least 6 weeks prior to recruitment. UV exposure within the 6 weeks preceding the study, pregnancy, or lactation were defined as exclusion criteria. The protocol (No. 14-111) was approved by the Ethics Committee of the University of Lübeck and all volunteers gave written informed consent beforehand.

2.2 | Irritants and mode of exposure

Aqueous solutions (50 μ L) of *n*-propanol (1-propanol; Merck, Darmstadt, Germany) in concentrations of 30.0%, 45.0%, 60.0%, or 75.0%, respectively, were applied on eight previously marked test fields on the upper-mid back on 4 consecutive days (D1-D4) two times daily for 30 minutes according to a recently validated occlusion-modified tandem repeated irritation test (om-TRIT) protocol.⁵ The irritants were applied to the respective test fields at the same time of the day (± 1 hour) using large Finn chambers (12 mm diameter; SmartPractice, Reinbek, Germany). On four of the test fields, exposure to the respective irritant concentrations was preceded by occlusion with distilled water for 30 minutes and on four fields the same irritant concentrations were applied without preceding occlusion; two adjacent fields, respectively, exposed to distilled water only (occlusion) or left untreated (normal skin) served as controls. The volunteers were

allowed to take a shower as usual, while the use of skin care products or UV exposure in the test area was not allowed for the entire duration of the study (5 days).

2.3 | Bioengineering assessment of the skin irritant response

Visual scoring, non-invasive assessment of erythema, transepidermal water loss (TEWL), and skin hydration (capacitance) were used to monitor the irritant response. The assessments were performed before the first application of the irritants (baseline) and 96 hours later (ie, at the end of the study), before tape stripping. Visual scoring was based on Kligman and Frosch, with assessment of erythema, scaling and fissuring based on a 0–4, respectively 0–3 point scale.⁷ Erythema was measured with the Colorimeter CL400 (CK Electronics, Cologne, Germany) and the values were expressed in the $L^*a^*b^*$ system; the a^* -value on the green-red axis was used for assessment of erythema. TEWL was measured with the open chamber system (Tewameter TM 300) and skin hydration was assessed by measuring capacitance (Corneometer CM 825; both devices from CK Electronics). For all parameters, two consecutive measurements per field were obtained by the same investigator under controlled environmental conditions (temperature $21^\circ\text{C} \pm 1^\circ\text{C}$; average relative humidity 40%–45%) and according to the published guidelines.^{8–11}

2.4 | Natural moisturizing factor analysis

The samples for stratum corneum natural moisturizing factor (NMF) analysis were taken 24 hours after the last irritant exposure on D5 using commercially available 14-mm D-SQUAME discs (CuDerm, Dallas, Texas) and stored in sterile 1.5-mL Eppendorf tubes (Eppendorf, Hamburg, Germany) at -80°C until analysis. Six samples per test field per volunteer were collected as previously described.¹² The stratum corneum NMF components (histidine, 2-pyrrolidone 5-carboxylic acid, *trans*- and *cis*-urocanic acid) on the tapes were extracted with 400 μ L of 25% (wt/wt) ammonia solution, evaporated to dryness and reconstituted in 200 μ L pure water before high-performance liquid chromatography-UV analysis.¹³ Extracts from two D-SQUAME discs were pooled for analysis; the NMF levels were corrected for the amount of protein and expressed as mmol NMF/g protein.

2.5 | Statistical analysis

GraphPad Prism Version 7 (GraphPad Software, San Diego, California) was used for statistical analysis. The level of significance was $P < .05$. Repeated-measures ANOVA or non-parametric Friedmann tests with additional correction for multiple comparisons (Dunn test) were used to evaluate the changes between the respective fields with regard to time and occlusion. The difference between AD and healthy controls was tested by one-way ANOVA with Bonferroni multiple comparison test or Kruskal-Wallis test in combination with Dunn multiple comparison test. Data in the respective tables and figures are presented as mean and SEM or as median with interquartile range, as indicated.

3 | RESULTS

The erythema (a^*), TEWL, and capacitance values at baseline and after 96-hour repeated exposure to the different concentrations of *n*-propanol with and without previous damage to the skin barrier by occlusion in the AD and healthy control groups are shown in Table 1. Within the study groups there were no significant differences in the visual irritation score and the barrier function parameters between the test and control fields at baseline.

3.1 | Repeated occlusion and water exposure modulate the concentration-dependent barrier damaging effects of *n*-propanol on healthy and atopic skin

Cumulative exposure to 45% *n*-propanol without previous barrier damage by occlusion led to significantly increased a^* -values in the AD group after 96 hours compared with baseline ($P < .01$). In contrast, at the same time point there were no significant changes in the a^* -values of test field exposed to the same irritant concentration in the healthy controls (Table 1). Previous barrier impairment by repeated exposure to water/occlusion enhanced the irritant-induced effects and led to a significant a^* -value increase after exposure to the lowest applied irritant concentration (30%) in both groups ($P < .05$ and $P < .01$ after 96 hours compared with baseline in the healthy control and AD groups, respectively). For both groups, no significant differences in the a^* -values following occlusion alone were found.

The relative changes of the a^* -values after 96-hour cumulative exposure to the different *n*-propanol concentrations, with and without previous occlusion, compared with baseline (Δa^*) are shown in Figure 1A. In both groups the a^* -value increased in parallel to the increasing applied irritant concentration. In the healthy controls, the differences in the Δa^* -values between the previously occluded and non-occluded fields were significant for all studied irritant concentrations ($P < .01$, $P < .0001$, $P < .01$ and $P < .001$ for 30%, 45%, 60%, and 75% *n*-propanol concentrations, respectively; Table 2).

Repeated occlusion with water alone resulted in impaired epidermal barrier function and significant TEWL increase on D5 compared with baseline in both groups ($P < .01$ and $P < .05$ in the AD and healthy control group, respectively; Table 1). In the AD group, cumulative exposure to 30% *n*-propanol alone was sufficient to induce a manifest damage to the epidermal barrier and the TEWL values of the respective test field on D5 were significantly higher compared with baseline (TEWL on D1 and D5 4.81 ± 0.19 and 6.66 ± 0.36 g/m²/h, respectively; $P < .001$). In contrast, in the healthy controls cumulative exposure to 30% *n*-propanol led to a significant TEWL increase only if the barrier function was previously compromised by occlusion/repeated water exposure. In both groups, TEWL after exposure to 45%, 60%, and 75% *n*-propanol was significantly increased on D5 compared with D1 and the observed effect was independent of barrier damage by previous occlusion with water (Table 1). Barrier damage prior to *n*-propanol exposure enhanced the irritant-induced effects and in the healthy controls the relative TEWL increase on D5,

assessed as Δ TEWL (Δ TEWL = TEWL D5 – TEWL D1), was significantly higher for all irritant concentrations (Table 2). In the AD group, the differences in Δ TEWL between the previously occluded and non-occluded fields on D5 were significant only after exposure to 30% and 45% *n*-propanol, respectively. In the presence of atopic skin disease, repeated exposure to *n*-propanol resulted in more severe impairment of the barrier function and on D5, Δ TEWL values of the test fields exposed to the same irritant concentration under the same conditions in the AD group were significantly higher than in the healthy controls (Figure 1B).

In both groups, cumulative exposure to all studied *n*-propanol concentrations with as well as without previous occlusion led to a significant decrease of capacitance of the respective fields on D5 compared with D1 (Table 1). The decrease in capacitance was concentration dependent in both the healthy and atopic groups as shown by the increasing Δ values parallel to the increase of the applied *n*-propanol concentration (Figure 1C). In AD, the Δ values on D5 compared with baseline were significantly greater if exposure to 45%, 60%, and 75% *n*-propanol was preceded by occlusion ($P < .01$, $P < .001$, and $P < .05$ for 45%, 60%, and 75% *n*-propanol, respectively). In the healthy controls the differences between the previously occluded and non-occluded fields exposed to the same irritant concentrations were significant only after exposure to 75% *n*-propanol (Table 2). The relative decrease of stratum corneum hydration on D5 was more pronounced in the healthy control group; the differences between the healthy and AD groups were significant for the test fields exposed to 45%, 60%, and 75% *n*-propanol with as well without previous damage by occlusion/water exposure (Figure 1C).

3.2 | Cumulative exposure to *n*-propanol reduces significantly the NMF levels in healthy and atopic skin

There were no significant differences in the baseline NMF levels between the groups. On D5, in both groups the NMF levels of all irritant-exposed fields, with as well as without previous damage by occlusion/water exposure, were significantly lower compared with the control fields (non-exposed/normal skin, respectively, occlusion with water); at the same time point there were no significant differences between the control fields (Figure 2). In the AD and the healthy control group, there were no significant differences in the relative reduction of the NMF levels between the previously occluded and the corresponding, non-occluded irritant-exposed fields. In contrast to the barrier function parameters, no significant differences in the relative NMF reduction of the test fields exposed to the same concentrations of the irritant under the same conditions between the healthy and the AD individuals were found.

4 | DISCUSSION

The aim of this study following the om-TRIT protocol was to investigate the relationship between the applied *n*-propanol concentration and the relative contribution of workplace-relevant co-exposures on

TABLE 1 Erythema (a*-value), transepidermal water loss (TEWL), and capacitance at baseline (D1) and after 96-hour (D5) repeated exposure to different concentrations of *n*-propanol (*n*-PrOH) with and without previous damage by repeated occlusion/water exposure in healthy controls (healthy; N = 20) and individuals with atopic dermatitis (AD; N = 20)

		Without previous damage by repeated occlusion/water exposure					With previous damage by repeated occlusion/water exposure				
		Ctrl	30% <i>n</i> -PrOH	45% <i>n</i> -PrOH	60% <i>n</i> -PrOH	75% <i>n</i> -PrOH	Occl. Ctrl	30% <i>n</i> -PrOH	45% <i>n</i> -PrOH	60% <i>n</i> -PrOH	75% <i>n</i> -PrOH
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
a*-value (AU)											
Healthy	D1	10.98 ± 0.36	11.38 ± 0.43	11.27 ± 0.45	10.94 ± 0.39	11.24 ± 0.37	11.30 ± 0.44	11.44 ± 0.45	11.13 ± 0.44	11.55 ± 0.40	11.28 ± 0.44
	D5	10.82 ± 0.39	11.57 ± 0.43	11.30 ± 0.44	11.60 ± 0.39*	12.08 ± 0.43*	11.88 ± 0.50	12.23 ± 0.45*	12.45 ± 0.36***	13.29 ± 0.42****	13.40 ± 0.58****
AD	D1	9.98 ± 0.31	10.37 ± 0.25	10.14 ± 0.27	10.02 ± 0.27	10.14 ± 0.27	10.01 ± 0.25	10.28 ± 0.29	10.17 ± 0.25	10.65 ± 0.32	10.49 ± 0.27
	D5	11.04 ± 1.15	11.70 ± 0.76	11.74 ± 0.62**	11.92 ± 0.62***	12.75 ± 0.70****	11.47 ± 1.04	11.96 ± 0.71**	11.97 ± 0.40***	13.33 ± 0.54****	12.93 ± 0.54****
TEWL (g/m ² /h)											
Healthy	D1	4.75 ± 0.30	4.83 ± 0.24	4.64 ± 0.27	4.58 ± 0.26	4.59 ± 0.26	4.45 ± 0.24	4.56 ± 0.25	4.32 ± 0.22	4.14 ± 0.20	4.26 ± 0.24
	D5	4.56 ± 0.24	5.29 ± 0.31	5.31 ± 0.33**	5.67 ± 0.29****	6.08 ± 0.31****	5.79 ± 0.31*	6.24 ± 0.30*	6.97 ± 0.35****	7.40 ± 0.35****	8.07 ± 0.47****
AD	D1	4.89 ± 0.21	4.81 ± 0.19	4.55 ± 0.19	4.72 ± 0.20	5.12 ± 0.25	4.36 ± 0.18	4.73 ± 0.18	4.34 ± 0.23	4.35 ± 0.19	4.41 ± 0.15
	D5	5.08 ± 0.21	6.66 ± 0.36***	8.24 ± 1.05****	10.56 ± 1.45****	12.12 ± 1.33****	6.35 ± 0.27**	9.10 ± 0.41*	11.86 ± 0.77****	13.37 ± 1.40****	13.67 ± 1.30****
Capacitance (AU)											
Healthy	D1	49.60 ± 1.53	48.14 ± 1.47	49.04 ± 1.53	49.38 ± 1.63	49.93 ± 1.50	49.76 ± 1.76	50.17 ± 1.59	50.55 ± 1.88	49.20 ± 1.71	49.56 ± 1.69
	D5	42.90 ± 1.81**	37.96 ± 1.79***	32.71 ± 1.74****	29.03 ± 1.63****	26.42 ± 1.34****	42.90 ± 1.81****	37.96 ± 1.79****	32.71 ± 1.74****	29.03 ± 1.63****	26.42 ± 1.34****
AD	D1	30.14 ± 1.65	29.41 ± 1.70	30.24 ± 1.60	30.19 ± 1.77	29.77 ± 1.77	30.35 ± 1.67	30.25 ± 1.74	29.56 ± 1.72	29.85 ± 1.74	30.11 ± 1.76
	D5	31.97 ± 1.67	23.23 ± 1.30***	21.74 ± 1.33****	20.40 ± 1.31****	19.19 ± 1.39****	27.94 ± 1.81	21.34 ± 1.30****	17.70 ± 1.31****	16.11 ± 1.43****	15.90 ± 1.47****

Abbreviations: AU, Arbitrary units; ctrl, non-exposed (normal skin) site; Occl. Ctrl, occlusion with distilled water.

Note: Data are presented as mean and SEM. Level of significance $P < 0.05$.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

**** $P < .0001$ compared with baseline (D1).

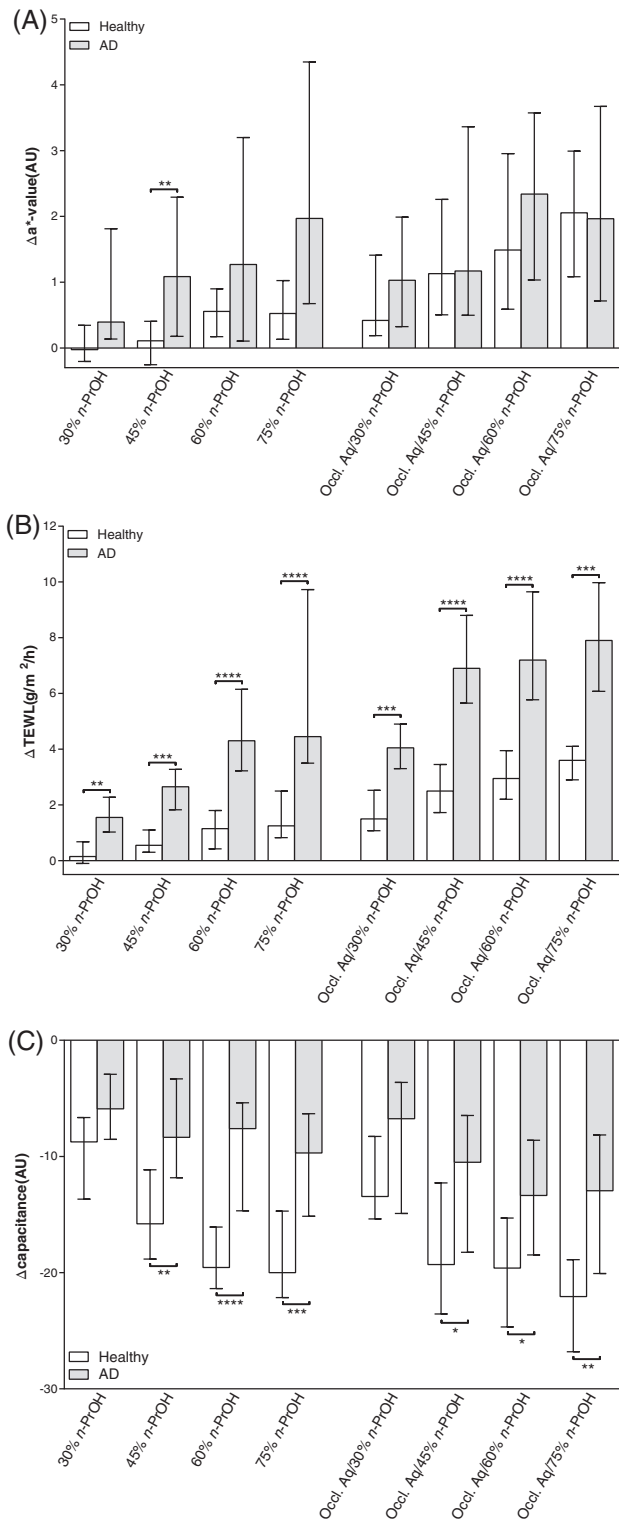


FIGURE 1 Comparison of the changes in the (A) a^* -values, (B) transepidermal water loss, and (C) capacitance after repeated exposure to different concentrations of *n*-propanol (30%–75%) with and without preceding damage to the epidermal barrier by occlusion/water in atopic (AD) and healthy individuals. The data are presented as Δ values (median and interquartile ranges) compared with baseline. Level of significance $P < .05$; * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$. AU, Arbitrary units; *n*-PrOH, *n*-propanol; Occl. Aq, occlusion with distilled water; TEWL, transepidermal water loss

the outcomes of cumulative exposure to the irritant in healthy and AD individuals. The results of the study show that the irritant and barrier damaging effects of *n*-propanol *in vivo* are dependent on the applied irritant concentration and are modulated by both exposure and host-related factors. In this line, whereas we found objectively measurable negative effects after exposure to even the lowest studied concentration, the relative changes in the inflammatory, barrier function, and biochemical parameters were more pronounced with increasing *n*-propanol concentrations. Furthermore, using an om-TRIT protocol, which was recently shown to differentiate the neat irritant-induced effects from the relative contribution of relevant co-exposures, we showed that repeated low-grade trauma by occlusion/water exposure alone impaired the barrier function and reduced the skin reactivity threshold to the irritant in both groups. As the cumulative duration of occlusion/water exposure under om-TRIT conditions corresponds to the definition of wet work, the findings of the study have important implications for development of irritant HE under real-life exposure conditions in both healthy and at-risk individuals.¹⁴ In the same context, the different outcomes of the same exposures in atopic and healthy skin, shown in the study, may contribute to both the risk and recalcitrance of irritant HE in AD. In agreement with our earlier publications based on exposure to 60% *n*-propanol, in this study we found reduced NMF levels after exposure to the entire range of investigated *n*-propanol concentrations and these findings were independent of the presence of atopic skin disease or previous damage by occlusion.

The number of published *in vivo* studies investigating the cumulative barrier damaging effects of repeated exposure to short-chain alcohols applied as single irritants has been limited. Lübke et al found no significant increase in TEWL after cumulative exposure to 60% *n*-propanol following previous damage to the epidermal barrier by overnight water exposure under occlusion in healthy skin.¹⁵ Based on these findings the authors suggested that the irritant potential of 60% *n*-propanol was comparable to water. Similarly, Löffler et al observed no significant changes in TEWL after repeated patch testing with ethanol, *n*-propanol, and isopropanol applied on healthy skin in concentrations ranging from 60% to 100%.¹⁶ In contrast, using a repeated open application test design, Clemmensen et al showed that neat *n*-propanol exerted an irritant effect comparable to that of 1.0% sodium lauryl sulfate (SLS) and underscored the importance of the test model used in experimental studies for prediction of skin irritation.¹⁷

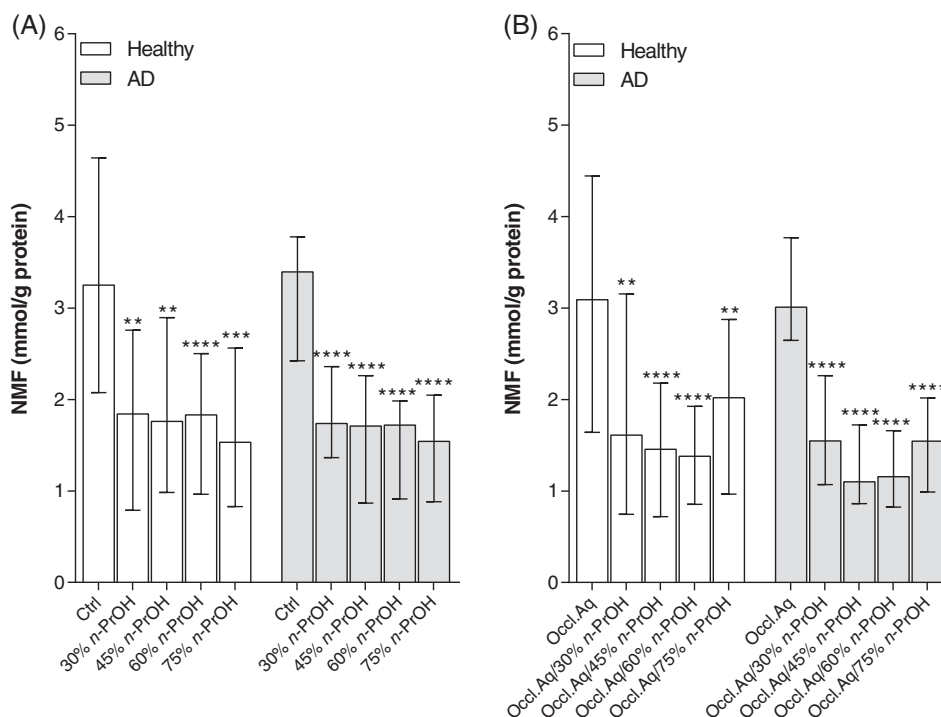
The changes in the barrier function parameters after cumulative exposure to 60% *n*-propanol in a TRIT in healthy volunteers were initially studied by Kappes et al.¹⁸ Using the same exposure model, we recently showed significant impairment of the permeability barrier function along with alterations of the corneocyte surface topography after exposure to the same concentration of the irritant and pointed to previously underestimated or unknown aspects of the irritant potential of short-chain alcohols.⁴ Furthermore, the findings of the present and our previous studies are in agreement with observations of an earlier om-TRIT study on the effects of 60% *n*-propanol and 0.5% SLS in the human skin *in vivo* as well as with the results of a recent controlled application test by Cartner et al, showing significant

TABLE 2 Differences in the Δ values of the test fields exposed to the same irritant concentrations with and without previous damage by occlusion/water exposure in the healthy and atopic dermatitis (AD) groups

n-PrOH concentration (%)	Δa^* -value (AU)		Δ TEWL (g/m ² /h)		Δ Capacitance (AU)	
	Healthy	AD	Healthy	AD	Healthy	AD
30	$P < .01$	NS	$P < .01$	$P < .01$	NS	NS
45	$P < .0001$	NS	$P < .0001$	$P < .0001$	NS	$P < .01$
60	$P < .01$	NS	$P < .0001$	NS	NS	$P < .001$
75	$P < .001$	NS	$P < .001$	NS	$P < .01$	$P < .05$

Abbreviations: AU, Arbitrary units; n-PrOH, n-propanol; NS, not significant; TEWL, transepidermal water loss.

Note: Level of significance $P < .05$.

**FIGURE 2** Stratum corneum natural moisturizing factor (NMF) levels after repeated exposure to different concentrations of *n*-propanol (30%-75%) (A) with and (B) without previous barrier damage by occlusion/water in atopic (AD) and healthy skin. Absolute values (median and interquartile ranges) are provided, with level of significance $P < .05$; ** $P < .01$, *** $P < .001$, **** $P < .0001$ compared with the non-exposed (normal skin) site. ctrl, Non-exposed (normal skin) site; n-PrOH, *n*-propanol; Occl. Aq, occlusion with distilled water

barrier function impairment after exposure to 70% *n*-propanol in healthy female volunteers.^{5,19} The pronounced irritant effect of *n*-propanol *in vivo* observed by Cartner et al was shown to translate *in vitro* to a marked cellular toxicity and significant tumor necrosis factor- α (TNF- α) release by the skin residential cell. These findings confirm earlier *in vitro* observations for significantly increased expression and release of key primary keratinocyte-derived cytokines, including interleukin-1 α (IL-1 α), TNF- α , and IL-6 in response to the irritant.^{20,21} Taken together, the aforementioned *in vivo* and *in vitro* investigations and our results provide solid evidence for the negative effects of *n*-propanol on the permeability barrier function and point to the contribution of short-chain alcohols to development of cumulative irritant HE in occupations with relevant workplace exposure.

Skin dryness and scaling are main and early signs of irritant HE. The maintenance of skin hydration depends on both the intercellular lipids which regulate the transport of water across the stratum

corneum and a mixture of low-molecular weight, water-soluble compounds, such as amino acids, organic acids, urea, and inorganic ions, collectively known as NMFs. Short-chain alcohols are penetration enhancers and known to exert pronounced effects on the intercellular lipids, including disruption of the lipid lamellae, lipid phase transition, and alterations in the lipid organization.²²⁻²⁸ Apart from these effects, short-chain alcohols, and in particular, *n*-propanol, were recently shown to reduce the activity of phospholipase A2 (PLA₂), one of the key enzymes involved in the maintenance of barrier homeostasis and lipid processing in the skin.¹⁹ In an earlier publication, we provided first evidence for significant reduction in the stratum corneum NMF levels after om-TRIT with *n*-propanol and/or SLS and demonstrated that short-chain alcohols may cause skin dryness through interaction with both the skin lipids and reduction of NMF.⁵ The results of the present study confirm and extend these observations by showing a significant decrease in the NMF levels after exposure to all tested *n*-propanol concentrations in both healthy and atopic skin. The

relative NMF reduction in the healthy controls ranged from 54.8% to 62.6% after exposure to the different irritant concentrations without previous barrier damage by occlusion and, from 46.3% to 61.6% if the corresponding fields had been previously exposed to water under occlusion. In the AD group, the relative NMF changes after irritant exposure without and with previous occlusion ranged between 46.2% and 61.4% and 42.3% and 54.3%, respectively. Importantly, similar to our previous om-TRIT study, occlusion with water alone did not have a significant impact on the NMF levels and these findings confirm that the observed effects in both groups were induced solely by the irritant. The significant NMF decrease after cumulative exposure to even the lowest (30%) *n*-propanol concentration and the lack of significant differences in the relative NMF changes between the lowest and the highest (75%) irritant concentration in both groups correspond to the desiccation effects reported under real-life exposure conditions and provide evidence for the contribution of a broad range of short-chain alcohol concentrations to the sum of low-grade events, leading to manifest irritant HE.

The pathomechanisms leading to NMF decrease after repeated exposure to *n*-propanol remain incompletely understood. Short-chain alcohols, including *n*-propanol, are known to exert protein denaturing and cytotoxic effects as well as compromise the epithelial cell membrane integrity which could facilitate the NMF escape from the cells.^{19,29–31} As more than 50% of the total NMF content is known to be derived by the proteolytic degradation of filaggrin, the recently shown decreased activity of the profilaggrin-processing enzyme kallikrein 5 (KLK5) by *n*-propanol, and to a lesser extent, isopropanol and ethanol *in vitro*, provide another possible explanation for our findings.¹⁹ Whether the previously reported, reduced activity of PLA₂ by *n*-propanol could negatively impact the maintenance of the acidic skin pH and thus affect filaggrin processing under om-TRIT exposure conditions *in vivo* remains speculative at present.

In contrast to the numerous epidemiological studies showing increased susceptibility to occupational irritant HE in the presence of atopic skin disease, there have been only few publications on the effects of cumulative exposure to single or multiple irritants under controlled exposure conditions in AD. The first evidence for significant impairment of the permeability barrier function after repeated exposure to 0.3 mL of 0.1% SLS, 2.3% disodium lauryl 3-ethoxysulphosuccinate, or 2.0% Shellsol K in atopic skin was provided by Tupker et al.³² Using a TRIT design, in an earlier study we showed that atopic skin is more susceptible to damage by even low concentrations of weak workplace irritants, such as 2% acetic acid, that would not exert a significant negative impact on healthy skin.³³ In addition, in another study based on the same model, we observed significant differences in the severity of the barrier function impairment and inflammatory response induced by repeated single and concurrent exposure to detergents and alkaline agents in AD.¹² In the present study we found that cumulative exposure to 30% *n*-propanol, applied as a single irritant, was sufficient to induce damage to the epidermal barrier in AD, whereas the same exposure had no significant effect on healthy skin, unless the barrier function had been previously impaired. The pattern of skin reactivity to cumulative exposure to

n-propanol was similar to the one observed in our previous studies and there were significant differences in the severity of barrier impairment of the test fields exposed to the same irritant concentration under the same conditions in the AD compared with the healthy control group. To the best of our knowledge, this is the first study on cumulative exposure to short-chain alcohols and at the same time first om-TRIT study in atopic skin, and its findings provide further experimental evidence for the increased susceptibility to irritant damage in AD.

The mechanisms underlying the more severe barrier impairment after cumulative irritant exposure in AD are incompletely understood, but could be attributed to both altered barrier properties and inflammatory responses in the presence of atopic skin disease. Compromised barrier function with increased baseline TEWL even in uninvolved skin is a major characteristic of AD and several groups in the past have shown a positive correlation between the pre- and post-exposure TEWL values after single as well as repeated SLS-induced irritation in both healthy and atopic individuals.^{32,34–37} Whereas these observations could partly explain our results, it remains controversial whether barrier responses to experimentally induced SLS irritation may predict the irritant responses to unrelated primary irritants, such as short-chain alcohols.³⁸

In addition to the increased baseline TEWL, the more severe barrier impairment after cumulative exposure to *n*-propanol shown in this study may be explained by earlier findings for increased percutaneous penetration of both hydrophilic and lipophilic compounds in AD. In this context, Jakasa et al observed significantly higher diffusion of SLS and polyethylene glycols of different molecular mass through clinically normal, uninvolved skin of AD volunteers as compared with healthy, non-atopic controls.^{39,40} Independently of Jakasa et al, an earlier study showed increased penetration of dimethyl sulfoxide and theophylline through the stratum corneum of AD patients.⁴¹ Using photoacoustic spectrometry, Hata et al found accelerated penetration of dyes of different water solubility such as Yellow 4 (hydrophilic) and Red 215 (lipophilic) in the clinically uninvolved skin of atopic volunteers.⁴²

Beyond the impaired barrier function and increased skin diffusivity, the findings of earlier studies showing that increased skin surface pH, as found in AD, results in the activation of proteases involved in the processing of the pro-forms of the IL-1 cytokines in the epidermis, provide another possible explanation for the enhanced barrier responses in atopic compared to healthy skin, observed in the present om-TRIT.^{35,43–45} As the volunteers in the study were not genotyped, our results do now allow for conclusions on the influence of *FLG* mutation carrier status on the outcomes of repeated exposure to *n*-propanol in atopic or healthy skin.

For correct interpretation of our findings, it is important to mention that the present study did not intend to analyze the cumulative effects of repeated exposure to short-chain alcohols in comparison with soaps or detergents, as these classes of irritants differ in terms of physicochemical properties, interact with different components of the epidermal barrier, and consequently exert distinct effects on the non-invasive parameters for assessment of the skin barrier function.

Although soaps and detergents maybe in general more irritating than alcohols, the multi-parametric approach with instrumental and biochemical parameters used in the present study provides solid evidence for significant barrier damaging effects of even low concentrations of *n*-propanol *in vivo*. The consistent effects found in the present and in our earlier investigations confirm the validity and reproducibility of the om-TRIT model for experimental studies on cumulative skin irritation in healthy and at-risk individuals. As in addition to *n*-propanol and mentioned, most of the marketed alcohol-based hand disinfectants contain also isopropanol or ethanol, it would be important to assess the cumulative effects of tandem exposure to different combinations of short-chain alcohols in future studies.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ORCID

Ivone Jakasa  <https://orcid.org/0000-0002-7961-4069>

REFERENCES

- Gold NA, Avva U. In: StatPearls, ed. *InternetAlcohol Sanitizer*. Treasure Island, Florida: StatPearls Publishing; 2018. <https://www.ncbi.nlm.nih.gov/books/NBK513254/>
- Kampf G, Keamer A. Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. *Clin Microbiol Rev*. 2004;17:863-893.
- Pedersen LK, Held E, Johansen JD, Agner T. Less skin irritation from alcohol-based disinfectant than from detergent used for hand disinfection. *Br J Dermatol*. 2005;153:1142-1146.
- Soltanipoor M, Stilla T, Riethmuller C, et al. Specific barrier response profiles after experimentally induced skin irritation *in vivo*. *Contact Dermatitis*. 2018;79:59-66.
- Angelova-Fischer I, Stilla T, Kezic S, Fischer TW, Zillikens D. Barrier function and natural moisturizing factor levels after cumulative exposure to short-chain aliphatic alcohols and detergents: results of occlusion-modified tandem repeated irritation test. *Acta Derm Venereol*. 2016;96:880-884.
- Williams HC, Burney PG, Hay RJ, et al. The UK working party's diagnostic criteria for atopic dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. *Br J Dermatol*. 1994;131:383-396.
- Frosch P, Kligman A. The soap chamber test: a new method for assessing the irritancy of soaps. *J Am Acad Dermatol*. 1979;1:35-41.
- Piérard GE. EEMCO guidance for the assessment of skin colour. *J Eur Acad Dermatol Venereol*. 1998;10:1-11.
- Pinnagoda J, Tupker RA, Agner T, Serup J. Guidelines for trans-epidermal water loss (TEWL) measurement. A report from the standardization Group of the European Society of contact dermatitis. *Contact Dermatitis*. 1990;22:164-178.
- Rogier V. EEMCO guidance for the assessment of transepidermal water loss in cosmetic sciences. *Skin Pharmacol Appl Skin Physiol*. 2001;14:117-128.
- Berardesca E. EEMCO guidance for the assessment of stratum corneum hydration: electrical methods. *Skin Res Technol*. 1997;3:126-132.
- Angelova-Fischer I, Dapic I, Hoek A-K, et al. Skin barrier integrity and natural moisturizing factor levels after cumulative dermal exposure to alkaline agents in atopic dermatitis. *Acta Derm Venereol*. 2014;94:640-644.
- Dapic I, Jakasa I, Yau NLH, Kezic S, Kammeyer A. Evaluation of an HPLC method for the determination of natural moisturizing factors in the human stratum corneum. *Anal Lett*. 2013;46:2133-2144.
- BAUA: Federal Institute for Occupational Health; TRGS 401 Risks Resulting from Skin Contact – Identification, Assessment, Measures 2011. https://www.baua.de/EN/Service/Legislative-texts-and-technical-rules/Rules/TRGS/pdf/TRGS-401.pdf?__blob=publicationFile&v=2. Accessed July 26, 2019.
- Lübbe J, Ruffieux C, van Melle G, Perrenoud D. Irritancy of the skin disinfectant *n*-propanol. *Contact Dermatitis*. 2001;45:226-231.
- Löffler H, Kampf G, Schmermund D, Maibach HI. How irritant is alcohol. *Br J Dermatol*. 2007;157:74-81.
- Clemmensen A, Andersen F, Petersen TK, Kalden H, Melgaard A, Andersen KE. The irritant potential of: a validation study of two different human *in vivo* test models: a validation study of two different human *in vivo* test models-propanol (nonanoic acid vehicle) in cumulative skin irritation: a validation study of two different human *in vivo* test models. *Skin Res Technol*. 2008;14:277-286.
- Kappes UP, Göritz N, Wigger-Alberti W, Heinemann C, Elsner P. Tandem application of sodium lauryl sulfate and *n*-propanol does not lead to enhancement of cumulative skin irritation. *Acta Derm Venereol*. 2001;80:403-405.
- Cartner T, Brand N, Tian K, et al. Effect of different alcohols on stratum corneum kallikrein 5 and phospholipase A2 together with epidermal keratinocytes and skin irritation. *Int J Cosmet Sci*. 2017;39:188-196.
- Ockenfels HM, Keim-Maas C, Funk R, Nussbaum G, Goos M. Ethanol enhances the IFN- γ , TGF- α and IL-6 secretion in psoriatic co-cultures. *Br J Dermatol*. 1996;135:746-751.
- Neuman MG, Haber JA, Malkiewicz IM, Cameron RG, Katz GG, Shear NH. Ethanol signals for apoptosis in cultured skin cells. *Alcohol*. 2002;26:179-190.
- Goates CY, Knutson K. Enhanced permeation of polar compounds through human epidermis. I. Permeability and membrane structural changes in the presence of short chain alcohols. *Biochim Biophys Acta*. 1994;1195:169-179.
- van der Merwe D, Riviere JE. Comparative studies on the effects of water, ethanol and water/ethanol mixtures on chemical partitioning into porcine stratum corneum and silastic membrane. *Toxicol In Vitro*. 2005;19:69-77.
- Merle C, Baillet-Guffroy A. Physical and chemical perturbations of the supramolecular organization of the stratum corneum lipids: *in vitro* to *ex vivo* study. *Biochim Biophys Acta*. 2009;1788:1092-1098.
- Hatta I, Nakazawa H, Obata Y, Ohta N, Inoue K, Yagi N. Novel method to observe subtle structural modulation of stratum corneum on applying chemical agents. *Chem Phys Lipids*. 2010;163:381-389.
- Thind R, O'Neill DW, Del Regno A, Notman R. Ethanol induces the formation of water-permeable defects in model bilayers of skin lipids. *Chem Commun (Camb)*. 2015;51:5406-5409.
- Moghadam SH, Saliaj E, Wettig SD, et al. Effect of chemical permeation enhancers on stratum corneum barrier lipid organizational structure and interferon alpha permeability. *Mol Pharm*. 2013;10:2248-2260.
- Horita D, Hatta I, Yoshimoto M, Kitao Y, Todo H, Sugibayashi K. Molecular mechanisms of action of different concentrations of ethanol in water on ordered structures of intercellular lipids and soft keratin in the stratum corneum. *Biochim Biophys Acta*. 2015;1848:1196-1202.
- Baker RC, Kramer RE. Cytotoxicity of short-chain alcohols. *Annu Rev Pharmacol Toxicol*. 1999;39:127-150.
- Strubelt O, Deters M, Pentz R, Siegers CP, Younes M. The toxic and metabolic effects of 23 aliphatic alcohols in the isolated perfused rat liver. *Toxicol Sci*. 1999;49:133-142.

31. McKarns SC, Hansch C, Caldwell WS, Morgan WT, Moore SK, Doolittle DJ. Correlation between hydrophobicity of short-chain aliphatic alcohols and their ability to alter plasma membrane integrity. *Fundam Appl Toxicol*. 1997;36:62-70.
32. Tupker R, Pinnagoda J, Coenraads P, Nater J. Susceptibility to irritants: role of barrier function, skin dryness and history of atopic dermatitis. Susceptibility to irritants: role of barrier function, skin dryness and history of atopic dermatitis. *Br J Dermatol*. 1990;123:199-205.
33. Angelova-Fischer I, Hoek AK, Dapic I, et al. Barrier function and natural moisturizing factor levels after cumulative exposure to a fruit-derived organic acid and a detergent: different outcomes in atopic and healthy skin and relevance for occupational contact dermatitis in the food industry. *Contact Dermatitis*. 2015;73:358-363.
34. Seidenari S, Guisti G. Objective assessment of the skin of children affected by atopic dermatitis: a study of pH, capacitance and TEWL in eczematous and clinically uninvolved skin. *Acta Derm Venereol*. 1995;75:429-433.
35. Ring J, Eberlein-König B, Schäfer T, et al. Skin surface pH stratum corneum hydration, trans-epidermal water loss and skin roughness related to atopic eczema and skin dryness in a population of primary school children. *Acta Derm Venereol*. 2000;80:188-191.
36. Werner Y, Lindberg M. Transepidermal water loss in dry and clinically normal skin in patients with atopic dermatitis. *Acta Derm Venereol*. 1985;65:102-105.
37. Agner T. Noninvasive measuring methods for the investigation of irritant patch test reactions. A study of patients with hand eczema, atopic dermatitis and controls. *Acta Derm Venereol*. 1992;173:1-26.
38. Björnberg A. Skin reactions to primary irritants in men and women. *Acta Derm Venereol*. 1975;55:191-194.
39. Jakasa I, de Jongh C, Verberk MM, Bos JD, Kezic S. Percutaneous penetration of sodium lauryl sulphate is increased in uninvolved skin of patients with atopic dermatitis compared with control subjects. *Br J Dermatol*. 2006;155:104-109.
40. Jakasa I, Verberk MM, Esposito M, Bos JD, Kezic S. Altered penetration of polyethylene glycols into uninvolved skin of atopic dermatitis patients. *J Invest Dermatol*. 2007;127:129-134.
41. Yoshiike T, Aikawa Y, Sindhvananda J, et al. Skin barrier defect in atopic dermatitis: increased permeability of the stratum corneum using dimethyl sulfoxide and theophylline. *J Dermatol Sci*. 1993;5:92-96.
42. Hata M, Tokura Y, Takigawa M, et al. Assessment of epidermal barrier function by photoacoustic spectrometry in relation to its importance in the pathogenesis of atopic dermatitis. *Lab Invest*. 2002;82:1451-1461.
43. Hosomi N, Fukai K, Nakanishi T, Funaki S, Ishii M. Caspase-1 activity of stratum corneum and serum interleukin-18 level are increased in patients with Netherton syndrome. *Br J Dermatol*. 2008;159:744-746.
44. Kezic S, O'Regan GM, Lutter R, et al. Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency. *J Allergy Clin Immunol*. 2012;129:1031-1039.
45. Proksch E. pH in nature, humans and skin. *J Dermatol*. 2018;45:1044-1052.

How to cite this article: Angelova-Fischer I, Soltanipoor M, Stilla T, Fischer TW, Kezic S, Jakasa I. Barrier damaging effects of *n*-propanol in occlusion-modified tandem repeated irritation test: Modulation by exposure factors and atopic skin disease. *Contact Dermatitis*. 2020;82:1-9. <https://doi.org/10.1111/cod.13368>