LITERATURE DATA

Glycerol

1. Hydrating effect of glycerol

The nasal mucosa is dehydrated in sinonasal conditions (common cold chiefly) where nasal discharge makes patients to blow frequently. In addition to discomfort, the dryness of the nasal mucosa compromises the mucociliary clearance of the airway that is an important self-defense mechanism playing an important role in the removal of foreign bodies [SUNWOO, 2006]. When this mechanism is impaired, the possibility of respiratory infections is increased. The influenza virus in the air loses infectivity in an environment with 50% RH and above, but its survival time is prolonged in an environment with an RH below 50% [*Harper*, 1963 cited in SUNWOO, 2006]. *Satsuta* [1985 cited in SUNWOO, 2006] reported that there is a seasonal factor in the rate of influenza infection, with respiratory infection prevalent especially in winter. Studies revealed that low atmospheric relative humidity conditions affected mucociliary clearance (Saccharine Clearance Time -SCT) of the nasal mucous membrane [SUNWOO, 2006]. Saccharine Clearance Time under 10% RH increased more than it did in the pre-room with 50% RH. An increase in Saccharine Clearance Time suggests a decrease in the activity of the mucociliary function.

Glycerol is a constituent that has long been used for its skin moisturizing effects. Relatively recent *in vitro* studies and *in vivo* studies in animals and human have evidenced the properties of glycerol with respect to the fluid balance. Glycerol is a 'wetting' agent. Due to its hygroscopic nature, glycerol is widely recognized for its ability to take up water from the external environment and from perspiration [FROEBE, 1990; CRICKX, 2002; LODEN, 2003; MARTINDALE Glycerol, 2005].

Thanks to the hygroscopic potential of glycerol, it can support the nasal mucosa hydration, relieve the discomfort related to the dryness and help the mucociliary clearance.

The effect of glycerol on nasal mucosa hydration has been studies in the following publication of which the characteristics are summarized in the table below:

Efficacy of glycerol on nasal mucosa hydration		
MIWA, 2006 Open, controlled study	44 healthy volunteers from 10 to 75 years old (average 45.5 ±17.2 years) 26 men and 18 women	0.9% NaCl solution 10% NaCl solution 10% glycerol solution Nasal barrier cream Single application of 100µL-5min

MIWA [2006] studied the effects of

- physiological saline,
- hypertonic saline (10% NaCl),
- **10% glycerol solution,**
- nasal barrier cream,

on nasal mucosal water loss (TEWL) in the basal state following non-traumatic application (**100µL-5min**) on the mucosal surface of the inferior turbinate in 44 adults and children. There were no volunteers who had obvious allergic rhinitis. Throughout the period of the study, there were no attacks of asthma or pollinosis. None of the subjects had used topical or systemic medication during the week before testing.
TEWL on the inferior nasal turbinate was measured with an evaporation meter applying Fick's law. Measurements (expressed in grams per square meter per hour) were made according to the European Group for Efficacy Measurements on Cosmetics and Other Topical Products guidance for the assessment of TEWL.
The results were statistically analyzed; Differences between control and test groups were tested using unpaired, two-tailed t-tests.

Basal TEWL values in male (36.4 ± 10.4 $g/m^2/h$) and female (35.7 ± 11.4 $g/m^2/h$) subjects were not different. The value tends to increase in order of age.
After application of

- 0.9% NaCl, TEWL decreased slightly but was not changed significantly (90.0 ± 12.3%; p = 0.151, as pretreatment control is 100%).
- 10% NaCl, TEWL was significantly increased compared with the basal state (140.9 ± 30.6%; p =┐ 0.008).
- nasal cream, TEWL was significantly decreased compared with basal state (74.6 ± 16.1%; p = 0.001).
- 10% glycerol, TEWL was also significantly lower compared with basal state (88.0 ± 13.6%; p = 0.006).

In conclusion, these results evidence the decreasing effect of the glycerol 10% solution on trans-epidermal water loss supporting its beneficial effect on the hydration of nasal mucosa [MIWA, 2006].

In addition to the well-known and recognized hygroscopic effect of glycerol [FROEBE, 1990; CRICKX, 2002; LODEN, 2003; MARTINDALE Glycerol, 2005], results of this study support the efficacy of glycerol for the hydration of nasal mucosa in the context of upper respiratory tract infections namely common colds/rhinopharyngitis.

2. Safety of glycerol

Glycerol is authorized as food additive (E422) in Europe without limitation for the acceptable daily intake [Additifs Alimentaires, 2016] and as an excipient of cosmetics and pharmaceutical products for infants, children and adults.
Glycerol is GRAS listed and included in the:

- FDA Inactive Ingredients Database (dental pastes; buccal preparations; inhalations; injections; **nasal** and ophthalmic preparations; oral capsules, solutions, suspensions and tablets; otic, rectal, topical, transdermal, and vaginal preparations).
- Non-parenteral and parenteral medicines licensed in the UK.
- Canadian List of Acceptable Non-medicinal Ingredients [ROWE, 2009].

Glycerol as excipient is used in a wide variety of pharmaceutical formulations including oral, otic, ophthalmic, topical, and parenteral preparations from 0.5 up to 80% [ROWE, 2009]. In topical pharmaceutical formulations and cosmetics, glycerol is used primarily for its humectant and emollient properties. When used as an excipient or food additive, glycerin is not usually associated with any adverse effects and is generally regarded as a nontoxic and nonirritant material.

Studies available in literature

VETTER [2012] showed that glycerol had no deleterious effects on ciliary beat frequency (CBF) and no toxicity on excised ciliated human nasal epithelial cells in *in vitro* study. The concentration-dependent effect of compounds including glycerol on CBF was assessed using a high-speed digital imaging method. Excised ciliated human nasal epithelial cells were incubated for 60 min with the compounds and determination of the half maximal inhibitory concentration (IC_{50}), followed by a reversibility test. Lactate dehydrogenase (LDH) test assessing the cell viability was performed on human nasal epithelial cells with the compounds. These were applied to nasal epithelial cells in IC_{50} values.

The IC_{50}, reversibility and cytotoxity are displayed in the table below.

Solubilizing agent	IC_{50} ± standard deviation (%)	Reversibility (%) at concentration $c = IC_{50}$	Cytotoxicity ± standard deviation (%) at concentration $c = IC_{50}$
Glycerol	55.2 ± 3.7 (v/v)	79.6 ± 4.8	1.8 ± 0.8
Propylene glycol	25.3 ± 4.9 (v/v)	94.2 ± 3.2	2.1 ± 0.8
Polyethylene glycol 300	24.6 ± 5.0 (v/v)	91.5 ± 3.8	3.8 ± 1.6
N,N-Dimethylacetamide	14.5 ± 3.2 (v/v)	68.5 ± 5.1	5.2 ± 1.2
Polyethylene glycol 400	13.8 ± 3.9 (v/v)	86.6 ± 3.0	4.9 ± 2.0
Ethanol	12.2 ± 5.0 (v/v)	89.4 ± 4.0	4.5 ± 1.2
Ethylendiamindihydrochloride	3.7 ± 1.1 (v/v)	78.0 ± 3.4	4.9 ± 1.8
Polyvinylpyrrolidon 25	3.6 ± 1.1 (m/v)	80.3 ± 5.3	3.6 ± 1.1
Polyvinylpyrrolidon 90	1.4 ± 0.3 (m/v)	80.5 ± 6.0	4.1 ± 1.1

IC_{50} values were calculated for each solubilizing agent by a polynomic function. Reversibility indicates the % of recovery of the CBF of ciliated cells 60 min after having washed out the

test solutions. Results from the LDH test of solubilizing agents, were applied to nasal epithelial cells at c = IC_{50} (means ± SD, n = 6).
The following rank order in IC_{50} values was obtained for the solubilizers: glycerol > propylene glycol > polyethylene glycol 300 > N,N-dimethylacetamide > polyethylene glycol 400 > ethanol > ethylendiamindihydrochloride > polyvinylpyrrolidon 25 > polyvinylpyrrolidon 90.
The % of reversibility was 79.6±4.8 for glycerol when tested at the IC_{50} (55.2±3.7%).
Results from the LDH test showed that human nasal epithelial cells treated with glycerol were characterized by only a slight decrease in viability and integrity (1.8±0.8%).
To note: the incubation time of 60 min was suitable for this study design. The normal mucociliary transit time in humans has been reported to be 12–15 min. In addition, the local toxicity of the different excipients measured by CBF *in vitro* is probably too sensitive. *In vitro*, the excised ciliated tissue is totally immersed in the test formulation, whereas *in vivo* the viable ciliated epithelial cells are protected by the physiological mucus barrier [VETTER, 2012].

MIWA [2006] evaluated the effects of 0.9% and 10% NaCl saline solutions, 10% glycerol solution and nasal barrier cream on nasal mucosal water loss (TEWL) in the basal state following non-traumatic application (**100µL-5min**) on the mucosal surface of the inferior turbinate in 44 adults and children. **The results evidenced the decreasing effect of the glycerol 10% solution on trans-epidermal water loss *versus* no effect of 0.9% NaCl solution supporting its beneficial effect on the hydration of nasal mucosa.**

Other studies evaluating the safety of glycerol are summarized in the table below:

Reference	***Subjects***	***Tested products/dose/duration***	***Results***
In adult population			
OJEDA, 2013 **Randomized, controlled, double blind parallel group multicentre study**	Adult patients with allergic rhinitis or rhinoconjonctivitis 53: micro-emulsion group 54: control group	Micro-emulsion: glycerol monooleate, propylene glycol, polyethylene glycol 400, sesame oil, polysorbate 80, sodium chloride 0.9%, menthol, eucalyptus oil and water, whereas the placebo was composed of sodium chloride 0.9%, menthol, eucalyptus oil and water Control: 0.9% NaCl solution 1 puff (50µL) per nostril, twice daily for 29 to 133 days	No serious adverse events occurred and most adverse events reported in both groups were related with the allergic rhinitis pathology in the form of nasal respiratory symptoms.

STOELZEL, 2014 **Randomized double blind placebo controlled study**	20 patienst wih allergic rhinitis 10: Nasya/Prevain spray group 9: control group	Nasya/Prevalin spray: bentonite, xanthan gum, glycerol monostearate, monopotassium phosphate, dipotassium hydrogen phosphate, glycerol anhydrous, sesame oil and spearmint oil Control isotonic sea water spray 280µL into each nostril, single dose	During the study, three mild AEs were documented in two subjects in the verum group (swallowing difficulties, nasal airways obstruction and headache); none related to the application of the investigational product. None of the AEs was classified as serious. There was no difference between the two treatment groups regarding the global assessment of tolerability provided by the investigators (P = 0.582) or by the subjects (P = 1.000). In 100% of the cases, the investigators rated the tolerability of Nasya/Prevalin or placebo with 'very good' or 'good'. Similar judgment was given by the subjects (Nasya/Prevalin: 90% 'very good' or 'good'; placebo: 100% 'very good' or 'good').

No adverse event was reported in these clinical efficacy studies for formulations containing glycerol.

3. REFERENCES

Literature

Additifs alimentaires: Glycerol (E422) available at http://www.additifs-alimentaires.net, 2016

Crickx B. Les émollients : lesquels, comment ? Nouv. Dermatol. 2002, 21: 25-26.

Froebe C.L., Simion A., Ohmeyer H. Prevention of *stratum corneum* lipid phase transitions in vitro by glycerol-an alternative mechanism for skin moisturization. J. Soc. Cosmet. Chem. 1990, 41: 51-65.

Loden M. The skin barrier and use of moisturizers in atopic dermatitis. Clinics in Dermatolog. 2003, 21(2): 145-157.

Martindale. Glycerol. The Extra Pharmacopoeia 2005.

Miwa M, Nakajima N, Matsunaga M, Watanabe K. Measurement of water loss in human nasal mucosa. Am J Rhinol. 2006 Sep-Oct;20(5):453-5.

Ojeda P, Piqué N, Alonso A, Delgado J, Feo F, Igea JM, Navarro A, Olaguibel JM, Subiza J, Nieto C, Andersson M. A topical microemulsion for the prevention of allergic rhinitis symptoms: results of a randomized, controlled, double-blind, parallel group, multicentre, multinational clinical trial (Nares study). Allergy Asthma Clin Immunol. 2013 Aug 27;9(1):32.

Rowe RC; Sheskey PJ, E Quin M. Glycerol, Handbook of Pharmaceutical excipients, 2009

Stoelzel K, Bothe G, Chong PW, Lenarz M. Safety and efficacy of Nasya/Prevalin in reducing symptoms of allergic rhinitis. Clin Respir J. 2014 Oct;8(4):382-90.

Sunwoo Y, Chou C, Takeshita J, Murakami M, Tochihara Y. Physiological and subjective responses to low relative humidity. J Physiol Anthropol. 2006 Jan;25(1):7-14.

Vetter A, Augustijns P, Bernkop-Schnürch A. Solubilizing agents in nasal formulations and their effect on ciliary beat frequency. Toxicol In Vitro. 2012 Feb;26(1):150-6.

