

## Short Term Scientific Mission (STSM) Report

### STSM details

<b>Title</b>	<b>Skin barrier assessment for workers handling drilling waste</b>
<b>Participant</b>	Jose Hernán Alfonso
<b>Host</b>	Sanja Kezic, Coronal Institute of Occupational Medicine, Amsterdam, The Netherlands
<b>Period</b>	24 – 31 March 2017



### Background

Scarce research within occupational dermatology has been performed in Norway previously. As a result of a productive participation in STANDERM, occupational dermatology has been included as a part of the new research strategy of the National Institute of Occupational Health in Norway.

In Norway, the oil and gas production generates a lot of waste yearly, which is shipped to land-based waste management plants for treatment, deposit or incineration. Oil and drilling waste was the greatest part of all hazardous waste collected in 2012, including drilling waste from the continental

shelf (Statistics Norway, 2012). Additionally, the amount of drilling waste will increase during the coming years according to a prognosis from the Norwegian Ministry of Petroleum and Energy.

In Norway, the management and recycling oil drilling waste is relatively new, and knowledge about occupational skin exposure and subsequent health problems among workers is lacking. According to the occupational health services, workers handling drilling waste report work-related skin problems frequently.

A project aimed to assess occupational skin exposure among these workers, effects of chemical exposure on the skin barrier, subsequent development of skin problems and mechanisms for individual susceptibility will contribute to identify targets for primary and secondary prevention.

This STSM is essential to get practice in techniques for skin barrier assessment that will be used in this post-doc project.

### Aim (purpose) of the STSM

The main goal of this STSM is to learn and get practice in quantitative methods to assess the skin barrier such as tape stripping and trans-epidermal water loss. These techniques will be used in my post-doc project titled: **“Skin exposure to oil and drilling products among workers handling drilling waste, exposure assessment, molecular biomarkers of skin exposure and early biomarkers of skin disease”**

1. To learn and get practice in the use of the tape stripping technique for determination of skin barriers components in the stratum corneum such as NMF, pro- and anti inflammatory cytokines.
2. To learn how to analyze tape stripping samples in the laboratory.
3. To learn and get practice in the use of quantitative techniques for the biophysical analysis of the stratum corneum (Trans-epidermal water loss, pH measurement, etc.).
4. To discuss *in vitro* models that may identify molecular biomarkers of exposure to drilling waste and early predictive biomarkers of skin disease.

### Work carried out during the STSM

#### Presentation of National Institute of Occupational Health, Oslo Norway

On the first day of this STSM, I presented the National Institute of Occupational Medicine in a plenary meeting at the Coronal Institute of Occupational Medicine, Amsterdam. I have also presented my post-doc project: Skin exposure to oil and drilling products among workers handling drilling waste. Exposure assessment, molecular biomarkers of skin exposure and early biomarkers of skin disease.

## Tape stripping

From day 1 to day 4 we focused on the first, second and third goal of this STSM.

- Protocol for tape stripping technique
- Critical review of the project protocol. How many tape strips should be taken? Which measurements should be included? Which instruments for trans-epidermal water loss, and tape stripping shall be bought? Time needed for each patient.

Stratum corneum depth is determined with measurement of optical density, based on the mass of the stratum corneum on each tape.



*Measurement of optical density*

## Analysis of tape strips

### Preparation of samples and extraction of NMF:

Two methods were compared: extraction of NMF with ammonia and with water. Extraction with ammonia takes approximately 6 hours, whilst extraction with water only 15 minutes. This research group is making a comparison study to assess whether the NMF extraction can be performed with just water.

### *Extraction with ammonia:*

One round includes extraction of NMF from the corneocytes by addition of ammonia, shaking for 2 hours and centrifuging with vacuum and heating. After the ammonia has evaporated, water is added and a small proportion of the sample is mixed with something called “Mobile fase” and run through the HPLC.

## Trans-epidermal water loss measurement

*Tewameter TM 300 (Courage & Khazaka)*



Trans-epidermal water loss was measured with Tewameter TM 300, which is an open chamber device. It measures transepidermal water loss based on diffusion principle/Ficks law where the difference in vapour pressure is related to evaporation rate of water. The main advantage of this device is the ability to do continuous measurements. Ambient humidity, ambient temperature and air convection are environmental factors that influence measurement. The model, calibration and probe pressure are also factors related to the device that can affect the measurements.

### *Measurements*

I have performed comparative measures for

1. Finger dermatitis of my own hand.
2. Skin without dermatitis
3. Skin before tape stripping
4. Skin after 3 times tape stripping
5. Skin after 6 times tape stripping
6. Skin after 9 times tape stripping



The open chamber has to be on the skin for at least 10 seconds (it can also be 20 sec or longer). For the purposes of this assay, we had the device on the skin for 45 seconds on each site. Four measurements for each site were performed and the average was calculated.

The curve shown on the screen became steady after some at least 10 seconds. It became steady quicker on the sites with finger dermatitis and skin barrier impairment (skin after 9 times tape stripping)

Figure 1 shows comparative TEWL of the hands with average measurements in g/hm<sup>2</sup> The highest TEWL measurements were found in finger dermatitis. Figure 2 shows comparative measurements of TEWL before and after tape stripping.

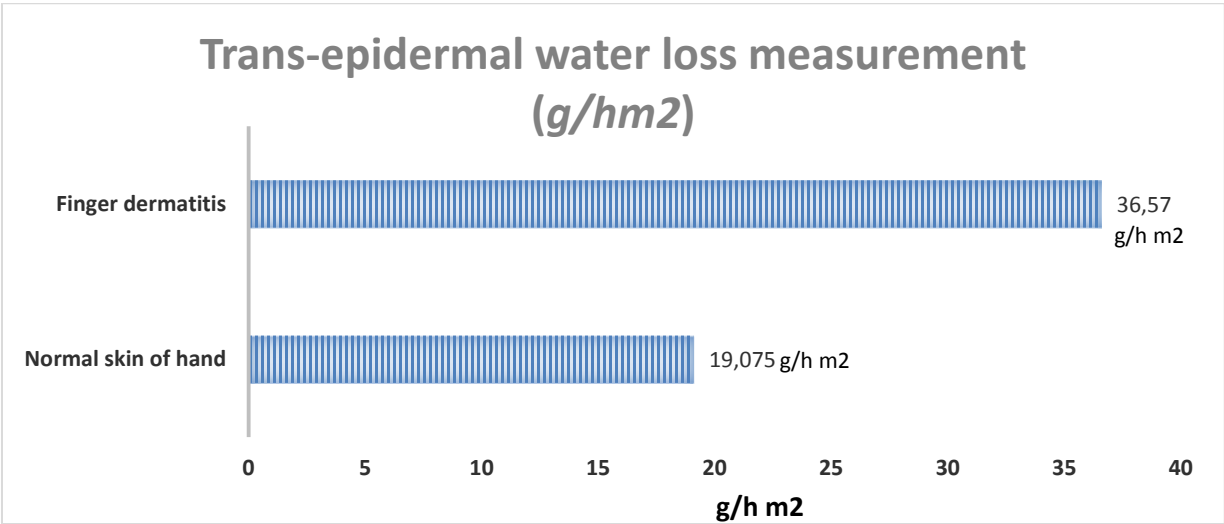


Figure 1. Comparative measurement of TEWL in finger dermatitis and normal skin of hand.

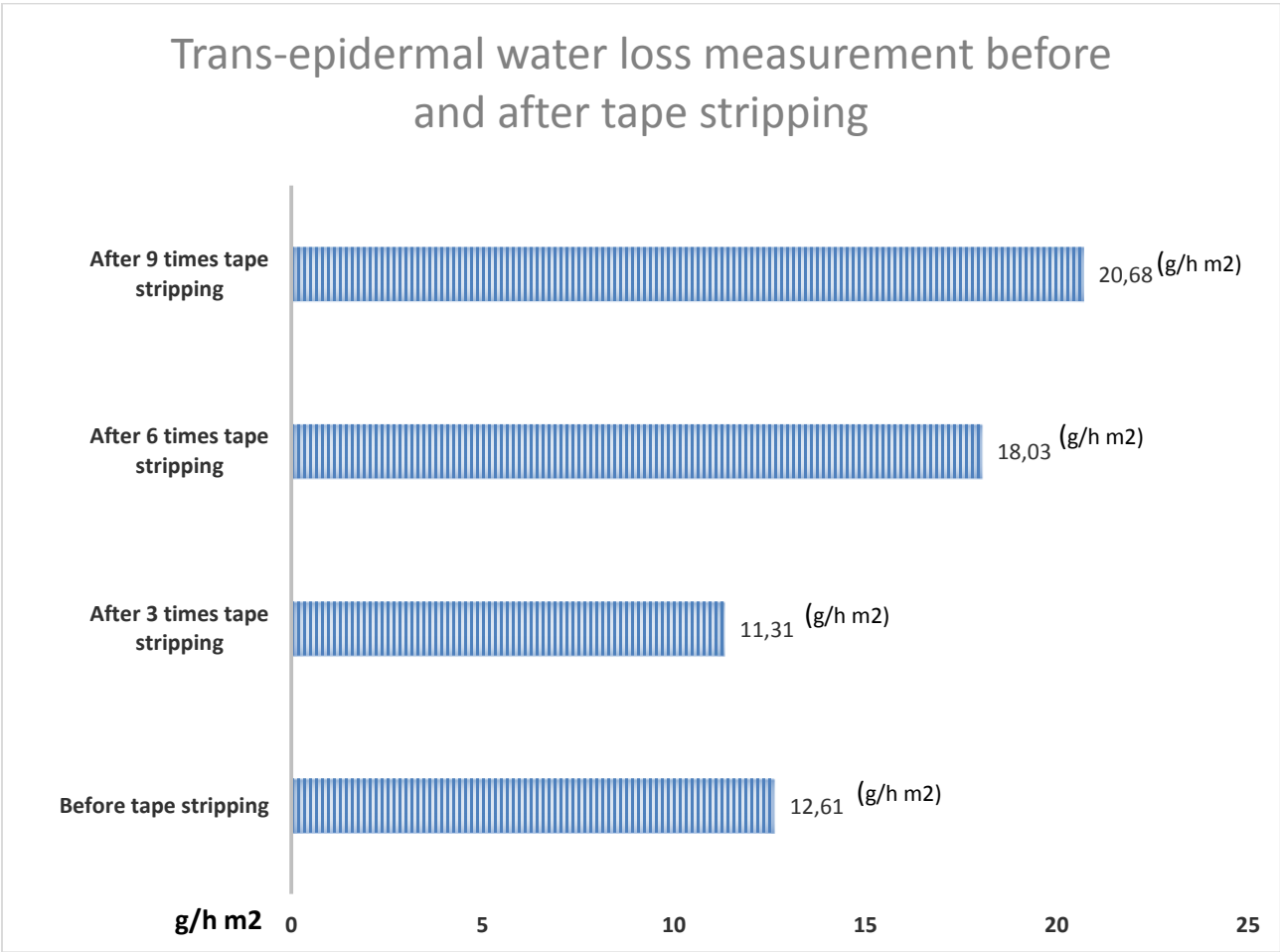


Figure 1. TEWL measurements before and after tape stripping

## In vitro models vs. In vivo models to assess skin barrier function



***Brainstorming about in vitro models vs. in vivo models to assess skin barrier function. From left to right: Sanja Kezic, Jose Hernan Alfonso and Shan Ziennoldinny***

Shan Ziennoldinny, senior researcher from the National Institute of Occupational Health visited also the Coronal Institute to discuss epigenetic models and in vitro models to determine skin barrier function.

We planned to establish a human organotypic cell culture model to study mechanisms of skin diseases related to the interaction between exposure (drilling waste) and the FLG gene (gene-work environment interactions).

The human organotypic cell model will consist of human fibroblasts purchased commercially and co-cultured with N/TERT keratinocytes (Harvard Medical School, USA). Human dermal equivalents will be generated as described by Commandeur S et al (2011) and Drongelen Vv et al (2013). The organotypic cell culture will be exposed to occupationally relevant doses of a typical compound present in the drilling waste (e.g PAH) and the same set of biomarkers as described in (3.1) will be measured. In addition, skin permeability will be measured using luciferin yellow, transepidermal water loss (TEWL), and keratohyalin granules will be analysed.

In separate experiments, a FLG gene will be knocked down (FLG-KD) using FLG-specific antiFLG SiRNA and exposed to the same dose of PAH and the molecular and cellular biomarkers measured as described above. For comparison, the effects will be measured in unexposed control cells.

Sanja Kezic recommended to focus on in vivo studies, as they are considered «gold standard» methods to determine skin barrier function. However, epigenetic interactions in the skin barrier function have not been analyzed so far and in vitro studies may be an alternative for hypothesis-testing. The main limitation of in vitro studies for assessing skin barrier function is the difficulty to interpret results that may have different directions and issues related to external validity.

After a productive discussion/brainstorming we decided to consider following mechanistic studies:

- Penetration of nanoparticles into the skin. Effect of nanoparticles on stratum corneum.
- Patterns of FLG expression according to pattern of exposures and skin response.

- Genotyping for IL-1alfa and IL-1 beta. Sanja can provide with authorisation for analyzing polymorphisms of IL-1alfa and beta in UK.
- For identification of biomarkers, consider to identify/compare the presence of specific biomarkers in blood, sputum and skin.
- Consider to take skin biopsy of workers with skin problems .
- For the in the depth mechanistics studies we need to identify one or several specific compounds from airborne and skin exposure.
- It would be interesting to focus on lipids as skin exposure to oils may extract constitutive lipids of the skin which have a role in the signalising of the immune response.

### **Working on research protocol**

Based on the new knowldege and practical training gained during this STSM, adjustments were made to the research protocol of my post-doc project.

Some of these adjustments include:

- Number of tape strips to take and localisation
- Storage of tape strips before measurement.
- Definition of mechanisms that the in-vitro study will focus on.
- Inclusion of a validated score to assess severity of hand dermatitis.
- Check lists to be included during field measurement.
- The in vitro part of the study will first analyse interactions between FLG gene and IL-1alfa and IL-1 beta.
- The National Institute of Occupational Health of Norway will attempt to isolate mRNA from tape stripping.

### **Main results obtained**

This STSM has been a great arena to increase networking and collaboration for future research on occupational exposures and skin barrier assessment in Norway.

All the goals have been fulfilled and future collaboration has been established.

I have gained training in techniques for skin barrier assessment that I will use in my post doc project.

### **Future collaboration with the host institution**

As a major result of this STSM, my post-doc project will include cooperation with highly reputed international research centers that have developed the analytical methods for skin barrier assessment and genotyping. In Netherlands, the Academic Medical Centre, Amsterdam (Prof. Sanja Kezic). In Croatia, the Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia (Prof. Ivone Jakasa). In Denmark: the National Allergy Research Centre- Copenhagen University

Hospital (Prof. Jacob P. Thyssen, MD Kristine Engebretsen). The last collaboration constitutes an extension of the collaboration established in a STSM in 2014, which was the basis of my Ph.D project. The tape strips from the skin barrier assessment of this post-doc project will be sent to the Coronel Institute for analysis of the degradation products of filaggrin and pro- and anti-inflammatory cytokines. Dr. Sanja Kezic will facilitate networking with K Bioscience UK for filaggrin genotyping and polymorphisms in gene of IL-1 alfa and IL-1 Beta.

#### Foreseen publications/articles resulting from the STSM

Three scientific publications are planned in the post-doc project **“Skin exposure to oil and drilling products among workers handling drilling waste, exposure assessment, molecular biomarkers of skin exposure and early biomarkers of skin disease”**. This project is entirely financed by the National Institute of Occupational Health, Oslo. Norway.

#### Other comments

I would like to greatly thank Sanja Kezic, Maryam Soltanipoor and the Institute Coronel of Occupational Medicine for this fruitful week.

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*March 31, 2017*