# Reactive oxygen stress generating capacity and inflammatory potential of settled dust samples from moisture damaged and reference schools

**Kati Huttunen[1,2], Jenni Tirkkonen[2], Martin Täubel[3], Juha Pekkanen[3], Dick Heederik[4], Jan-Paul Zock[5,6,7], Anne Hyvärinen[3], Maija-Riitta Hirvonen[1], Kelly BéruBé[1]**

*[1]Cardiff University, School of Bioscience, Cardiff, United Kingdom; [2]University of Eastern Finland, Department of Environmental Science, Kuopio, Finland; [3]Institute for Health and Welfare, Department of Environmental Health, Kuopio, Finland; [4]Utrecht University, Institute for Risk Assessment Sciences, Division Environmental Epidemiology, Utrecht, The Netherlands; [5]Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain; [6]Universitat Pompeu Fabra (UPF), Barcelona, Spain; [7]CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain*

## AIMS

Exposure to moisture damaged indoor environment is associated with adverse respiratory health effects, but responsible factors remain unidentified. In order to elucidate the mechanism behind these effects, Reactive Oxidative Stress (ROS)-generating capacity of settled dust samples (n=25) collected from moisture damaged and reference schools in Spain, The Netherlands and Finland was evaluated. In addition, the results were compared with immunotoxicological endpoints analysed with an *in vitro* model.

## METHODS

The settled dust samples were collected to cardboard boxes for 8 weeks from reference (n=11) and moisture damaged (n=14) schools from Spain, The Netherlands and Finland. Sample was vacuumed from the cardboard box onto MCE-filter, suspended to diluting buffer and stored in a freezer. Samples of each school were pooled, filtered, aliquotted and frozen again before further analysis.

ROS capacity was assessed with a plasmid scission assay (PSA), which determines the dose able to damage 50 % of DNA from a plasmid sensitive to ROS ($TD_{50}$ value).

Immunotoxicological endpoints such as production of inflammatory markers as well as mitochondrial activity, viability, apoptosis and cell cycle arrest were analysed *in vitro* after exposing mouse RAW264.7 macrophages to settled dust sample for 24 hours.

## RESULTS

The average $TD_{50}$ values showed that samples from The Netherlands had higher ROS capacity (= lower $TD_{50}$ values) compared to samples from The Spain and Finland (Fig. 1). The results were in line with the findings of an *in vitro* model showing higher producton of inflammatory mediators and toxicity of the Dutch samples, although the difference between $TD_{50}$ values of samples from moisture damaged and reference environments was not statistically significant (Fig.2). However, the results of the mouse macrophage model indicated that in two out of three countries, the immunotoxic potency of samples was higher in moisture damaged environments (Fig. 3).

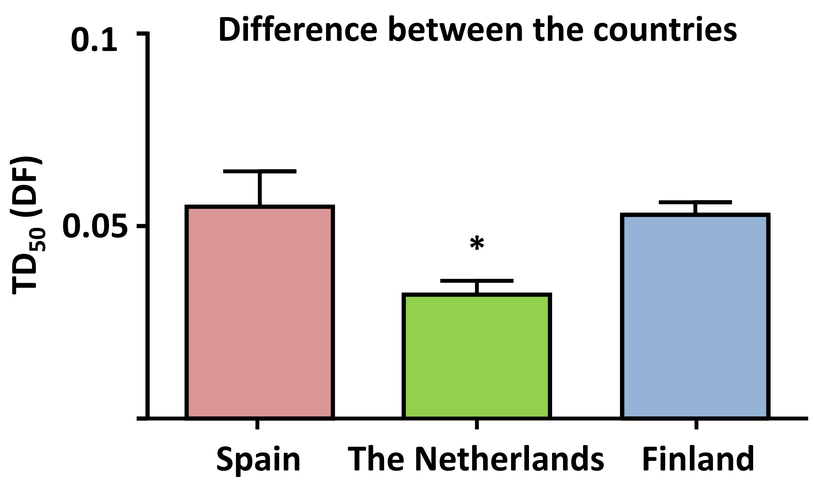


***Figure 1.*** *Comparison of $TD_{50}$-values (mean ± SEM) of settled dust from schools in Spain, The Netherlands and Finland. TD = median toxic dose, DF = dilution factor, * = statistically significant difference, p<0.05*

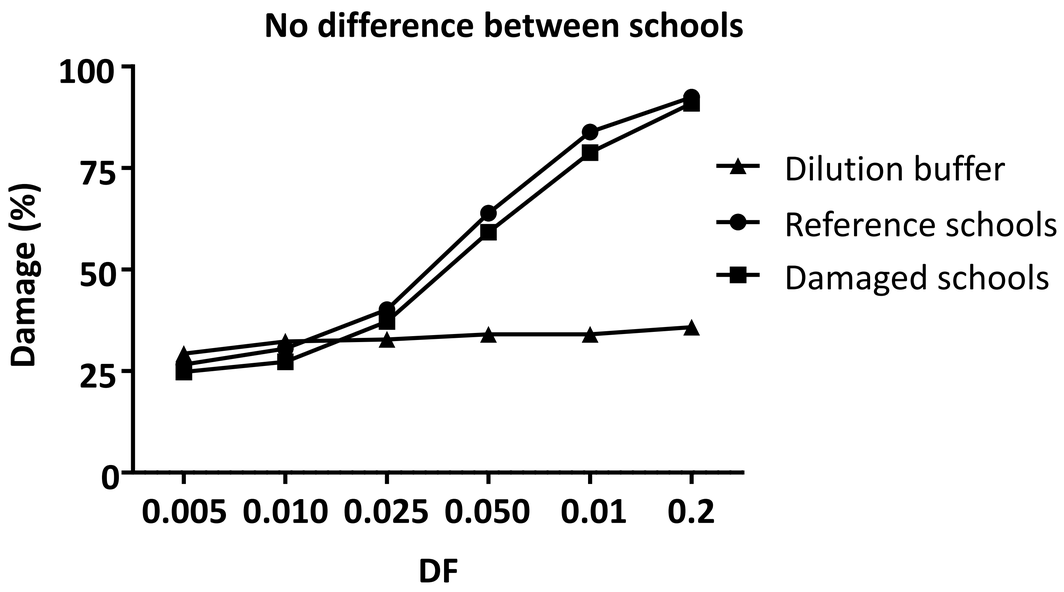


***Figure 2.*** *Comparison of dose-response curves of plasmid DNA damage caused by control exposure (dilution buffer) and settled dust from moisture damaged and reference schools. DF = Dilution factor*

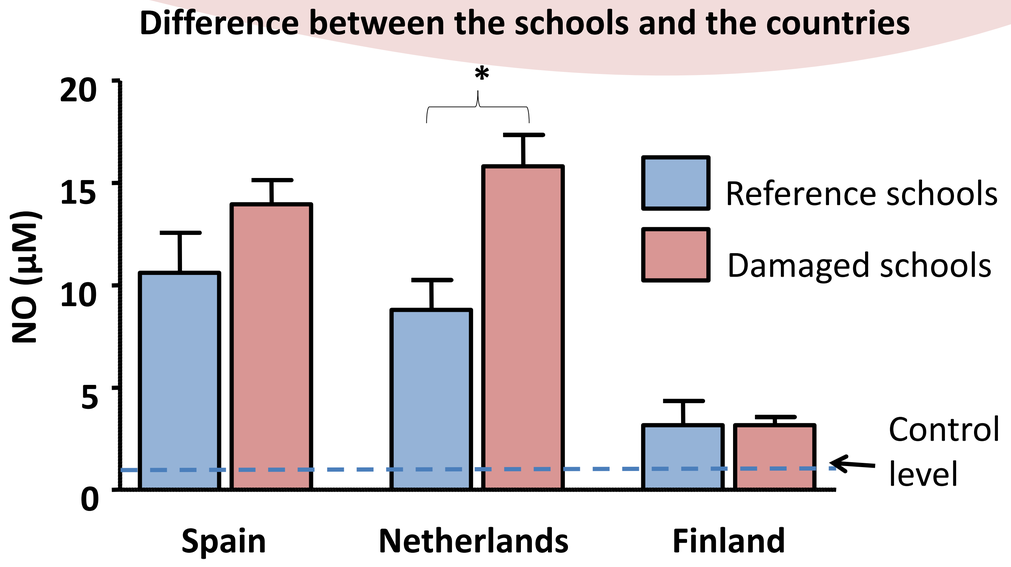


***Figure 3.*** *Comparison of NO-production (mean ± SEM) of settled dust from moisture damaged and reference schools in Spain, The Netherlands and Finland. * = statistically significant difference, p<0.05*

## CONCLUSIONS

The results indicate that the ROS generating capacity, along with immunotoxicological activity of settled dust differs between geographical locations. The inflammatory potential of dust tends to be higher in moisture damaged buildings, but geographical differences and high variance confounds the differentiation between moisture damaged and reference environments.

## ACKNOWLEDGEMENTS

We acknowledge the support of the European Respiratory Society, Fellowship LTRF 2013 – 1505 and the support of the European commission as part of HITEA (Health Effects of Indoor Pollutants: Integrating microbial, toxicological and epidemiological approaches).

ERS EUROPEAN RESPIRATORY SOCIETY every breath counts

LUNG and PARTICLES RESEARCH GROUP

CARDIFF UNIVERSITY PRIFYSGOL CAERDYDD

UNIVERSITY OF EASTERN FINLAND

HITEA