



HELIOS

QLK4-CT-1999-01308

**BIOMARKERS FOR THE NON INVASIVE ASSESSMENT OF ACUTE
AND CHRONIC EFFECTS OF AIR POLLUTANTS ON THE
RESPIRATORY EPITHELIUM**

**Development and Application to Adults and Children along a North-South European
Gradient**

Summary of Final Report

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Summary

Over the last decades, epidemiological and experimental studies have clearly shown that ambient air pollutants can produce a variety of short-term adverse health effects, including decrements of lung function, lung inflammation, increased airways permeability, exacerbation of pre-existing diseases such as asthma and even premature cardio-vascular mortality in the most vulnerable groups. Although long-term effects of air pollution are much less documented, there is growing evidence from both epidemiological and animal studies that chronic exposure to air pollutants is detrimental to the lung tissue and can contribute to the risks of some respiratory diseases including asthma.

Current research in the biomarkers field is opening new perspectives with the development of molecular tools that enable the non-invasive measurement of the extent of lung inflammation or damage without any major restriction related to exposure conditions, age or health status of examined subjects. These new tools have been used in the HELIOS project in order to assess the short- or long-term effects of air pollutants while exploiting the range of ozone (O₃) pollution existing along the North-South European gradient. These tests were applied in children in particular who are among the most vulnerable population. These studies were also carried out with the aim to test several mechanistic hypotheses (lung hyperpermeability and blood hypercoagulability hypotheses) that could explain some associations between air pollution and health effects and perhaps provide some clues for the largely unexplained rise in respiratory diseases such as childhood asthma.

Long-term effects of air pollutants. Chronic effects of air pollution were studied by comparing lung biomarkers and spirometric tests between children and adults living along a North-South European gradient characterised by intermediate concentrations of O₃ in ambient air. The extreme points were provided by the city of Parma, in Northern Italy and by the city of Umea in Northern Sweden whereas the intermediate point was provided by Brussels or Lille. In healthy children, the HELIOS partners found there was no statistical difference to be observed either in the lung function parameters or in the serum levels of pneumoproteins along the North-South European gradient. The levels of these parameters were remarkably similar in the children recruited in Sweden, Belgium and Italy, and small variations observed for some indicators were unrelated to the North-South gradient, disappearing completely after adjustment for confounders such as age or body mass index. Although these observations were based on relatively small cohorts, they do not provide evidence of chronic toxic effects on the lung function or epithelium that could be attributed to differences in air quality along the European gradient, in particular with respect to O₃ levels.

However, studies carried out in Brussels and in Umea led to the discovery of a so far unsuspected air pollutant, closely related to our Western lifestyle, which can be a major determinant of the lung epithelium integrity of children. This pollutant is trichloramine, an irritant gas building up in the air of indoor chlorinated pools. Although this gas can reach concentrations that are way above those of classic air pollutants (with mean levels of around 500 µg/m³ in Belgian pools), its health impact has remained almost totally unevaluated over the past decades. The HELIOS project first showed that, above a certain concentration (300 µg/m³), this gas can acutely and chronically damage the children's lung epithelium, and second, that regular exposure to this gas and possibly also to chlorinated aerosols was indeed associated with an increased risk of developing atopic asthma. These challenging observations have paved the way to the chlorine hypothesis, which might contribute to the rise in childhood asthma observed in industrialised countries, particularly in those countries with a cold climate

where most pools are indoors (Ireland, Scotland, Canada, ...). The HELIOS project indicates thus that there is an urgent need for further investigations of the chronic effects of this gas, for the implementation of preventive measures and in priority for the determination of an air quality standard concerning trichloramine.

Short-term effects of air pollutants. The HELIOS project provides evidence of a threshold for the short-term effects of ozone (O_3) in ambient air. The measurement of lung function and of sensitive indicators of lung inflammation or damage in non exercising and non asthmatic children exposed to increasing levels of ambient O_3 did not reveal any sign of lung inflammation, oxidative stress or functional impairment below a level of $100 \mu\text{g}/\text{m}^3$. The earliest adverse effect consisting in a marked elevation of exhaled nitric oxide (NO) was found from an ozone level (Benchmark dose) of $135 \mu\text{g}/\text{m}^3$ for the maximal 1-hour O_3 concentration or of $110 \mu\text{g}/\text{m}^3$ for the maximal 8-hour O_3 concentration. These thresholds were unaffected by the sex or age of the children and appear to be similar in adults. Signs of oxidative stress were also found in particular in exhaled breath condensates of adults exposed to ambient O_3 levels higher than $160 \mu\text{g}/\text{m}^3$. Changes in lung epithelium permeability and in lung function were not detected in non-exercising children exposed to O_3 levels up to $230 \mu\text{g}/\text{m}^3$ and thus appear to require higher doses of inhaled O_3 . A significant increase in serum CC16 was found in exercising adults exposed to $400 \mu\text{g}/\text{m}^3$ in the HELIOS inhalation chamber studies whereas for ambient O_3 , the protein was found to rise above an ozone level of $180 \mu\text{g}/\text{m}^3$ for exercising adults as reported in the FP4 PNEUMO-NEPHROTOX project. O_3 appears to be particularly effective in increasing the lung epithelium permeability compared to particulate matter. Whereas serum CC16 was found to rise rapidly during short-term exposure to O_3 , no change was detected in the level of the protein following exposure to relatively high concentrations of either foundry dust or diesel exhaust gases. By contrast, chronic exposure to foundry dust was associated with a decreased concentration of the protein, which probably reflects a concomitant destruction of Clara cells similar to that seen with tobacco smoke.

Observations made in the HELIOS project do not provide evidence of an activation of the blood coagulation system by ambient air pollutants, at least at moderate exposure levels, even in particularly sensitive subjects. However, the possibility that this endpoint may be affected at very high exposure levels or following more sustained exposures can not be excluded. It may be that activation requires a combination of conditions that were not reproduced or encountered during the HELIOS project (morbid conditions, high temperature, combination of high levels of ozone or particulate matter in the air...etc). Further studies are needed to test this hypothesis.

New non-invasive tests of lung inflammation or damage. The results obtained during the HELIOS project confirm the great potential of new non invasive tests of pulmonary damage or inflammation based on serum or exhaled air to assess the health risks of air pollutants. Because of their non-invasiveness and easy repeatability these tests can be used in vulnerable subjects such as children, even in field conditions. For short-term effects, they enable to refine dose-response relationships since the same subjects can be examined shortly before and after the episode of air pollution, making the assessment both more sensitive and reliable. The HELIOS project has further validated the assay of serum CC16 as a sensitive indicator of lung epithelium damage caused by ambient air pollutants such as ozone. The inhalation chamber study has clearly shown that, provided diurnal variations are taken into account, the concentration of CC16 in serum can be used to detect increases in lung epithelium permeability induced by O_3 . By using proteome analysis, the HELIOS project also identified

new potential biomarkers of oxidative stress or lung damage in exhaled breath condensate or among lung secretory proteins such as the haptoglobin 1 isoform, the carbonyl adducts to plasma proteins or the UGRP-1 pneumoprotein.

New etiological and mechanistic hypotheses for childhood asthma rise. The HELIOS project brought new insight into the hygiene hypothesis, which was raised more than 15 years ago, uncovering a new facet that so far had remained largely unexplored. The project shed a new light onto the topic of exposure to chemicals such as chlorine and its derivatives in particular, which are now indeed more widely used than ever in order to achieve hygiene standards. These chemicals, to which our children are increasingly exposed, might, as suggested by the HELIOS project, interact with allergens and/or some genetic traits to increase the risk of developing asthma. Further studies should be undertaken in order to elucidate the precise mechanisms by which chlorine-derived oxidants could sensitise the children's airways to asthma and in particular to further test the chlorine/atopy interaction, which, if confirmed, could be a preventable cause of the current childhood asthma epidemic affecting industrialised countries. An attractive hypothesis examined during the course of the project is the lung "*hyperpermeability*" hypothesis according to which ozone or other air pollutants would disrupt the lung epithelium barrier, thereby facilitating the loss of lung secretory proteins and/or the penetration into the lung of inhaled particles or plasma proteins. If particles trapped in the subepithelial space have allergic properties, they might elicit and perpetuate an immune response leading to allergic reactions. Further research in that area should exploit the new non-invasive techniques of lung inflammation/damage (exhaled breath condensate, exhaled NO, nasal NO, pneumoproteins levels, ...) to obtain objective and measurable indicators of exposure or conditions predisposing to childhood asthma. These new tools and concepts could also prove useful in assessing the contributory role, if any, of other air pollutants, such as ozone, particulate matter and chlorine-based oxidants for example, in the rise of this increasingly common disease.

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