5th Framework Programme

Quality of Life and

Management of Living Resources

Key Action 4

Environment and Health

Project No QLK4-2000-00573

RISK ASSESSMENT OF WOOD DUST: ASSESSMENT OF EXPOSURE, HEALTH EFFECTS, AND BIOLOGICAL MECHANISMS

Acronym: WOOD-RISK

FINAL REPORT

Reporting Period for the Final Report:

01/03/01 – 30/11/04

Contract number: QLK4-2000-00573
(include reference to complementary contracts-e.g. fellowships, INCO)

Title of the project: Risk Assessment Of Wood Dust: Assessment Of Exposure, Health Effects And Biological Mechanisms
(as in the contract)

Acronym of the project: WOOD-RISK
(as in the contract)

Type of contract: RTD
(E.g. RTD project, demonstration project…)

QoL action line:
(state to which key action, generic activity etc this contract belongs)

Commencement date: 01/03/01; Extension granted 03/10/03
(DD/MM/YY: normally the first day of the month following the day of the signature of the last contracting party, unless otherwise stated in the contract)

Duration: 45
(in months)

Total project costs: 2,428.002
(in Euro)

EU contribution: 1,350.000
(in Euro)

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Keywords:Wood dust, exposure assessment, inflammation, cancer, database
*(list **up to five** keywords that best describe the project)*

World wide web address:www.ttl.fi
(Internet address where updated information on the project can be obtained)

List of participants:
(provide same details as for the co-ordinator)

- **Partner 2**: Institut National de Recerche et de Securité (INRS), Vandeouvre, FRANCE; Principal Investigator: Dr. Raymond Vincent
- **Partner 3**: National Institute of Occupational Health (AMI), Copenhagen, DENMARK; Principal Investigator: Prof. Håkan Wallin
- **Partner 4**: Institut National de la Santé et de la Recherche Médicale (INSERM), Saint-Maurice, FRANCE; Principal Investigator: Dr. Danièle Luce
- **Partner 5**: Ludwig Maximilians Universität München, Munich, GERMANY; Principal Investigator: Prof Friz Krombach

(ii) Participants

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(iii) Scientific entities and teams involved

Exposure assessment and WOODEX/WOODRISK database

Partner 1, Scientific team

Assessment of occupational exposure to wood dust was carried out by two teams at the Finnish Institute of Occupational Health (FIOH): (i) Dr. **Timo KAUPPINEN** (PhD, responsible scientist; email timo.kauppinen@ttl.fi), and Mr. **Antti KAUPPINEN**, Dept. Epidemiology and Biostatistics, FIOH, Helsinki; (ii) Dr. **Irma WELLING** (PhD, responsible scientist; email irma.welling@ttl.fi), Ms. **Tuula LIUKKONEN**, MSc, and Ms. **Heini HONKANEN**, MSc, Lappeenranta Regional Institute of Occupational Health (LRIOH), Lappeenranta.

Partner 2, Scientific team

Exposure assessment carried out at Institut National de Recherche et de Sécurité (INRS), Département Métrologie des Polluants, Laboratoire de Caractérisation du Risque chimique, Vandoeuvre, France, has been carried out by the following team: M. **Raymond VINCENT** (Chemical engineer, principal contractor correspondent; email raymond.vincent@inrs.fr), M. **Michel GRZEBYK** (Statitistician), and Mrs. **Barbara SAVARY** (Chemical Engineer).

Experimental studies on pulmonary inflammation

Partner 1, Scientific team

The experimental studies on pulmonary inflammation have been carried out at FIOH by the following team: Prof. **Kai SAVOLAINEN** (MD, PhD, coordinator of the consortium, responsible scientist for the studies on pulmonary inflammation; email Kai.Savolainen@ttl.fi), Prof. **Harri ALENIUS** (PhD, Head of Unit of Excellence on Immunotoxicology; email Harri.Alenius@ttl.fi), Dr. **Marja-Leena MAJURI** (PhD, Senior Scientist), Mr **Juha MÄÄTTÄ** (MSc, PhD student); Dr. **Lea PYLKKÄNEN** (PhD, research scientist), Ms **Sari TILLANDER** (chief technician), Ms **Helene STOCKMANN-JUVALA**, (MPharm, PhD student), Ms. **Heidi HAATAJA** (lab. technician). Dr. **Timo TUOMI** (PhD, senior scientist, Dept. Occupational Medicine), and Mr. **Jaakko SÄNTTI**, (MSc, research scientist; Aerosol Laboratory) have also contributed to the study. In addition, Mr. **Pasi HYNYNEN** (MSc, research scientist), Ms. **Sari RAUTIO** (MSc eng, research engineer), Mr.**Timo MIELO** (Laboratory engineer), and Mr. **Pertti NÄRHI** (Chief technician), all from Lappeenranta Regional Institute of Occupational Health, Lappeenranta, were involved in the work.

Partner 5, Scientific team

The work has been carried out at Ludwig-Maximilians-Universitaet, Muenchen; Institut fuer Chirurgische Forschung, Muenchen; Germany, and the scientific team has included Prof. **Fritz KROMBACH** (responsible scientist for the pulmonary inflammation studies in Germany; email krombach@med.uni-muenchen.de), Dr

Huayan LONG (scientist), Ms **Anne-Marie ALLMELING** (technician), and Ms **Silvia MUENZING** (technician).

Sino-nasal cancer study

Partner 1, Scientific team

The sino-nasal cancer study has been carried at the Finnish Institute of Occupational Health (FIOH), Helsinki. The research team has included: Prof. **Kirsti HUSGAFVEL-PURSIAINEN** (PhD, co-coordinator of the WOOD-RISK project, Head of the FIOH Action Programme on Wood Dust: Exposure and Health Effects, and responsible scientist for the SNC study in Finland; email Kirsti.Husgafvel-Pursiainen@ttl.fi), Ms. **Reetta HOLMILA**, (MSc [eng.], PhD student, FIOH Research and Action Programme on Wood Dust), Ms. **Tuula SUITIALA** (chief technician, FIOH Research and Action Programme on Wood Dust), Dr. **Henrik WOLFF** (MD PhD, pathologist, Helsinki University Hospital, and FIOH), Ms. **Satu-Marja SNELLMAN** (MSc, programme secretary, FIOH Research and Action Programme on Wood Dust), Dr. **Pirjo HEIKKILÄ** (PhD, senior scientist, Dept. Epidemiology and Biostatistics), Ms. **Ritva DEGERTH** (MSc, industrial hygienist, Uusimaa Regional Institute of Occupational Health, Helsinki), Ms. **Tuula LIUKKONEN** (MSc, industrial hygienist, Lappeenranta Regional Institute of Occupational Health, Lappeenranta) and **Marja-Liisa LINDBOHM**, (PhD, senior scientist, Dept. Epidemiology and Biostatistics). The study has involved continued collaboration with Dr. **Eero PUKKALA** (PhD, Chief Statistician) from the Finnish Cancer Registry (subcontractor).

Partner 3, Scientific team

The team responsible for the study in Denmark has involved at the National Institute of Occupational Health, Copenhagen, following persons: Prof. **Håkan WALLIN** (PhD, responsible scientist for the SNC study in Denmark; email hwa@ami.dk), **Jette BORNHOLDT-LANGE** (Scientist, PhD student), and **Lourdes M. PEDERSEN** (Lab technician). In addition, major contributors of the study include Dr. **Johnni HANSEN (**PhD, Senior researcher), Danish Cancer Registry, **Troels BUNDGGARD** (MD, Dr. Med.), Head Neck Surgery, Aarhus University, **Michael DICTOR** (MD, Med. Dr.), Dept of Pathology, University of Lund, **Torben STEINICHE** (MD, Dr. Med.), Pathology, Aarhus University, and **Annemarie ANTONSEN** (MD, Dr. Med), Dept of Pathology, Roskilde Amtssygehus.

Partner 4, Scientific team

The study has been carried out at the Occupational and Environmental Epidemiology Unit, Institut National de la Santé et de la Recherche Médicale (INSERM), by the following team: Dr. **Danièle LUCE** (PhD, Senior Scientist, responsible scientist for the SNC study in France;; email Daniele.Luce@st-maurice.inserm.fr), and Ms. **Annie SCHMAUS** (research technician). In addition, Ms **Joëlle FÉVOTTE** (industrial hygienist), Institute of Occupational Medicine, Lyon, has contributed to the study.

(iv) Summary

The various activities of the WOOD-RISK project carried out during the years 2000-2006 are summaried in the following sections.

Assessment of occupational exposure to wood dust

The exposure assessment activities of the WOOD-RISK project aimed at estimating occupational exposure to inhalable wood dust by country, industry, the level of exposure and type of wood dust in 25 member states of the European Union (EU-25) for the purposes of hazard control, exposure surveillance, and serving as basis for assessment of health risks.
National labour force statistics, a country questionnaire (in 15 member states, EU-15), a company survey (in Finland, France, Germany and Spain), exposure measurements (from Denmark, Finland, France, Germany, the Netherlands and the United Kingdom), and expert judgments were used to generate preliminary estimates of exposure to different types of wood dust. The estimates were generated according to industrial class (6 wood industries, 4 other sectors) and level of exposure (5 classes). These estimates were reviewed and finalised by national experts from 15 member states. Crude estimates were generated also for 10 new member states (EU-10).
In 2000-2003, about 3.6 million workers (2.0% of the employed EU-25 population) were occupationally exposed to inhalable wood dust. Of those, construction employed 1.2 million exposed workers (33%), mostly construction carpenters. The numbers of exposed workers were 700,000 (20%) in the furniture industry, 300,000 (9%) in the manufacture of builders' carpentry, 200,000 (5%) in sawmilling, 150,000 (4%) in forestry, and <100,000 in other wood industries. In addition, there were 700,000 exposed workers (20%) in miscellaneous industries employing carpenters, joiners, and other woodworkers. The numbers of exposed workers varied by country ranging from <3,000 in Luxembourg and Malta to 700,000 in Germany.
The highest exposure levels were estimated to occur in the construction sector and furniture industry. Due to limited exposure data there was considerable uncertainty in the estimates concerning construction woodworkers. About 560,000 workers (16% of the exposed) may be exposed to a level exceeding 5 mg/m^3. Mixed exposure to more than one species of wood and dust from wooden boards was very common, but reliable data on exposure to different species of wood could not be retrieved. This kind of assessment procedure integrating measurement data, company data, country-specific data, and expert judgment, could also serve as one model for the assessment of other occupational exposures.
The basic data and final estimates have been launched as printed WOODEX reports and WOODEX database currently available on CD, and distributed via the network of national contact person created during the course of the project.

Wood dust-induced pulmonary inflammation

Respiratory symptoms, impaired lung function, and asthma have been reported in workers exposed to wood dust in a number of epidemiological studies. The underlying pathomechanisms, however, are not well understood. By the secretion of various cytokines, alveolar macrophages and type II lung epithelial cells are involved in the initiation and modulation of the inflammatory reaction in the lung.

In this project, we have studied (i) the induction by wood dusts of inflammatory response and the release of reactive oxygen species (ROS) in rat alveolar macrophages and lung epithelial lung cells in vitro, (ii) wood-dust induced expression of proinflammatory chemokines and cytokines in murine alveolar macrophages in vitro and in mouse model in vivo; (ii) induction of genotoxicity and inflammatory cytokines by various wood species in parallel experiments in human lung epithelial cells in vitro; and (iv) effects of wood dusts on apoptosis and ROS generation in human bronchial epithelial cells in vitro.

Inflammatory response in primary alveolar macrophages. The *in vitro* data from experiments carried out using primary rat alveolar macrophages and lung epithelial cells from indicate that (i) dusts from various hardwood and softwood species as well as dust from MDF are able to induce the release of proinflammatory mediators in primary alveolar macrophages by a mechanism that is, at least in part, mediated by ROS and (ii) the release of proinflammatory mediators from wood dust-exposed macrophages may be important in stimulating cytokine release from lung epithelial cells, thus amplifying the inflammatory response.

Cytokine and chemokine expression in murine macrophages and in vivo mouse model. Results from our present *in vitro* investigations demonstrate that wood dust is able to induce a heterogeneous expression of cytokines and chemokines in mouse macrophage RAW 264.7 cell line. The effects of the two studied wood dust groups, softwood and hardwoods, on the cytokine and chemokine expression of RAW 264.7 cells appear to be rather similar. However, some differences were detected between different wood dust species in their ability to affect cytokine and chemokine expression in RAW 264.7 cells. Oak dust was weaker inducer of cytokine and chemokine expression than birch dust in RAW 264.7 cells. On the contrary, in vivo, in mice, in the presence of all resident lung cells, repeated oak dust exposure induced stronger cytokine and chemokine expression than birch dust. Our results suggest that both hardwood and softwood dusts can influence the development of the inflammatory process through macrophages by modulating the expression of macrophage derived cytokines and chemokines, which are the key molecules in the regulation of leukocyte recruitment to the site of inflammation. Our *in vivo* studies demonstrate that repeated exposure to wood dust can elicit lung inflammation in mice. Both oak and birch dusts are able to induce the recruitment of macrophages, lymphocytes, neutrophils, and eosinophils into the lungs of mice. Oak and birch dust exposure elicited quantitative and qualitative differences in pulmonary inflammation suggesting that different wood species may also have differences in elicitation of inflammatory responses. Our in vivo studies demonstrate also that repeated exposure to wood dust is able to modulate allergic asthma. Exposure to oak dust modulates allergic asthma at least in two ways in mice: on one hand by inducing TNF-α expression in lung tissue, increasing the number of macrophages in the lungs, and increasing serum IgG2a levels, and, on the other hand, by suppressing methacholine-induced bronchial responsiveness and inhibiting expression of IL-13 and several chemokines which are typical for the Th2-type immune response of allergic asthma.

Genotoxicity and cytokine response in human lung epithelial cells. Our in vitro studies carried out with human lung epithelial cells show that exposure to wood dust resulted in DNA strand-breaks in four out of seven wood dusts examined. DNA strand breaks occurred before the inflammation had reached its maximum, which suggests that wood dusts themselves may have genotoxic potential. The distribution of the species that caused strand breaks, and those that did not, was

randomly distributed between the hardwood and softwoods. Cytokine responses were induced by all seven species. The lowest and highest responses were found among the hardwoods, and the responses of the softwoods were between the extremes found among hardwoods.

Apoptosis and ROS generation in human bronchial epithelial cells. The present findings in human bronchial epithelial cells *in vitro* show that exposure to wood dust activate ROS production and that they have an effect on both necrotic and programmed cell death on human bronchial epithelial cells. These results are well in line with the data obtained from other cell types and cell lines exposed to same types of wood dusts in the WOOD-RISK project.

In overall summary, these data generated by the WOOD-RISK project collectively suggest an elevated risk of pulmonary disorders due to repeated exposure to wood dust, whether from hardwood or softwood species, mediated via inflammatory mechanisms.

Molecular epidemiology study on sino-nasal cancer

In addition to a wide variety of acute and chronic non-malignant respiratory health effects, wood dust exposure is known to be associates with malignant diseases, cancer of the nose and paranasal sinuses in particular. The role of occupational exposure to wood dust in causation of sino-nasal cancer has long been established by numerous epidemiological studies, with the highest risks, in some earlie studies extremely high ones, observed for hardrwood dusts and adenocarcinoma histology. Mechanisms by which exposure to wood dust increases the risk of sino-nasal cancer (SNC) are nevertheless largely unclear. The present study involved a large collection of SNC tumours, by far the largest one studied so far to our knowledge, collected in three Eurpean countries (Denmark, Finland and France) which vary in major types of woodworking industries and principle types of wood species used. The tumours included in the study underwent a common review of histo-pathological diagnoses carried out by a panel of pathologists.

Our present results of the on-going study revealed a very high frequency of *TP53* mutations (70-80 %) in sino-nasal tumours. The mutation frequencies and types showed variation according to country, based on the preliminary analysis. Despite the high prevalence of adenocarcinoma histologies among SNCs, e.g. more than 50 % in France, the frequency of *KRAS* mutations remained low. Additional immunohistochemistry analyses were also performed. Completion of data on occupational exposure histories and tobacco smoke exposure will allow analysing possible role of occupational wood dust exposure in SNC tumorigenesis. Moreover, case-control studies are being carried out in France, Denmark and Finland to generate overall risk estimates for SNC in association to wood dust exposure in these countries. In summary, based on our current molecular analysis, it appears apparent that mutational mechanisms are frequently involved in tumourigenesis process in SNC.

All in all, the WOOD-RISK project has been highly successful in completing a series of biomedical studies exploring cellular and molecular mechanisms by which wood dust may induce health effects such as airway inflammation and cancer.

Exploitation and dissemination of results

In addition to the scientific activities, the WOOD-RISK project has paid a special emphasis on exploitation and dissemination of the results of the project, not only to a special emphasis on exploitation and dissemination of the results of the project, not only to the experts within the scientific community via conference

presentations and scientific publications, but also to occupational safety and health personnel in enterprises, governmental authorities and regulators, as well as to workplaces and laypeople in different member states. Indeed, the project has been active each year in organizing workshops, meetings or training courses for dissemination of practical information of exposure and health effects of wood dusts.An effort that goes markedly beyond the goals and responsibilities of an EU FP funded project is the activity of the Consortium to disseminate and exploit the results of the project to have a positive impact on the society, both at the Community and EU Member State level. These activities are exemplified by the major role of the WOOD-RISK project in organising the Wood Dust Symposium 2004 in Copenhagen, Denmark, and an international scientific and stakeholder congress Wood Dust Conference 2006 to be held in Strasbourg, France.