

<b>NOT CONFIDENTIAL</b>		
<b>Title of the project</b> MECHANISMS OF TOXICITY OF ASBESTOS-SUBSTITUTE MINERAL FIBRES: NEW APPROACHES TO HAZARD AND RISK ASSESSMENT		
<b>Acronym of the project</b> FIBRETOX		
<b>Type of contract</b> SHARED COSTS		<b>Total project cost</b> 1,800,080 €
<b>Contract number</b> QLK4-CT-1999-01629	<b>Duration</b> 44 Months	<b>EU contribution</b> 1,188,210 €
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## Section 2: Project Progress Report

### **NOT CONFIDENTIAL**

#### **Objectives:**

Mineral fibres are important industrial materials with an extensive range of applications and a wide potential for human exposure. While the use of asbestos is prohibited in Europe because of its proven ability to cause lung disease (including cancer), many other kinds of fibres are in use. Consequently there is a continuing need for improved understanding of the mechanism of fibre toxicity in order to provide scientific support for relevant regulatory actions. The main objective of the FIBRETOX project was the investigation of the mechanisms of toxicity of mineral fibres, focusing particularly on the mechanism of genotoxicity (a phenomenon related to carcinogenesis), with the ultimate aim to strengthen the scientific basis for hazard and risk assessment.

Three fibres of varying chemical composition and biopersistence (rockwool-type Japanese standard fibre RW1, glass fibres MMVF10 and wollastonite) were selected as study models and their effects were examined and compared to those of South African amosite asbestos fibres with proven carcinogenic potential. A basic question addressed concerned the importance indirect, inflammation-mediated versus direct pathways of genotoxicity, a question with important implications for risk assessment. For this purpose, the induction of a series of events related to lung inflammation, oxidative stress and genotoxicity was examined in animal systems. To obtain further mechanistic insights, analogous studies were conducted *in vitro* using primary cultures of rat lung epithelial type II cells and lung macrophages.

Additional issues addressed in the project concerned a) the possible interaction between fibres and benzo[a]pyrene (a tobacco smoke carcinogen) in the induction of genotoxic effects, in view of the known carcinogenic synergy between asbestos and tobacco smoke, and b) the use of biomarkers to assess exposure to, or early effect of, fibres in humans. Biomarkers are chemicals or cellular components which can be measured in human fluids or tissues and can be used to evaluate exposure to, or early biological effects of, environmental toxicants.

## Results and Milestones:

In order to investigate the pathways leading to genotoxicity in the lung, the test fibres were administered intra-tracheally to F344 rats, and a large number of markers reflecting lung toxicity, inflammation, immunotoxicity, oxidative stress, oxidative damage to DNA and other cellular components, as well as gene- and chromosome mutagenesis, were evaluated at different times after treatment. The markers evaluated included, among others, the composition of the fluid lining the inner surfaces of the lung as well as the lung tissue itself (type and number of cells, levels of pro-inflammatory molecules such as chemokines, interleukins, TNF $\alpha$  and NFkappaB, cell proliferation-inducing cytokines, molecules and biochemical activities reflecting the efficiency of cellular antioxidant defences), different types of DNA and chromosomal damage, DNA repair activity, and tissue pathology. Furthermore, an appropriate animal system (F344-derived lambda-IaI transgenic rats) was utilized to investigate the induction in the lung by three of the test fibres (not wollastonite) of gene mutations, a phenomenon of relevance to carcinogenesis.

It was found that all four fibres could induce changes in early markers of lung inflammation. However, only amosite and RW1 induced changes suggestive of persistent inflammation which could be observed up to 16 weeks post-treatment. Such prolonged inflammatory activity was associated with increased levels of DNA damage and lung mutagenesis (observed only with these two fibres). These events are believed to have been the result of a prolonged flux of tissue- and DNA-damaging reactive oxygen and nitrogen species (oxidative stress) generated by chronic inflammation. In contrast, MMVF10 induced less inflammation but no mutagenesis, while wollastonite caused only minor and short-lived inflammatory changes. The fact that no mutagenesis was caused by MMVF10, despite the generation of significant amounts of DNA damage, implies that additional factors were required for mutagenesis, probably related to the induction of cell proliferation, as suggested by the ability of amosite and RW1 to cause substantial increases in the levels of proliferation-inducing cytokines.

Analysis of the dependence of induction of chronic inflammation, oxidative stress and genotoxicity on the type of fibre employed, the doses administered and the time since animal treatment, provided evidence for a causal link between these events. Furthermore, given that the iron content of amosite was much higher than that of RW1, direct formation of cell-damaging, reactive oxygen species by chemical reactions catalysed by iron seemed unlikely to explain the mutagenic effects of these two fibres. Therefore it seems likely that a direct genotoxicity mechanism did not play a major role and that chronic inflammation, leading to persistent oxidative stress was the most important mediator of genotoxicity.

Further evidence regarding the mechanism of induction of genotoxicity by the test fibres was sought through studies utilising lung alveolar macrophages and type II epithelial cells freshly isolated from rat lungs. In addition, a mixed system was employed, consisting of macrophages cultured in proximity to, but not in direct contact with, the epithelial cells. By examining, in the latter system, the effects caused in epithelial cells by chemicals released upon treatment of macrophages with fibres, information on analogous effects occurring during inflammation in the animal lungs could be obtained.

A large number of parameters related to cell toxicity, genotoxicity and oxidative stress were examined in these studies. All fibres were found to affect some of the markers examined, especially in alveolar macrophages, with amosite and RW1 affecting the largest number of markers. One of the most interesting observations made, using the co-contact co-culture system, related to the ability of these two fibres to substantially upregulate in epithelial cells, upon treatment of macrophages, the expression of iNOS, a gene with a key role in the regulation of the cell cycle but also for the production of reactive nitrogen species which can lead to genotoxicity. This correlates with the distinct genotoxic behaviour of these two fibres and provides additional support for the importance of inflammation in the induction of genotoxicity.

Turning to the investigation of interactions between fibres and benzo[a]pyrene, simultaneous intra-tracheal administration to rats of benzo[a]pyrene with amosite or RW1 (but not MMVF10) was found to result in substantially higher lung mutagenesis as compared to the effects of the two agents separately, implying a potential for synergistic interaction of these fibres with tobacco smoke.

Finally, in order to evaluate the utility of biomarkers in the assessment of human exposure to, or early effects of, fibres, studies were conducted on workers from 3 factories in Slovakia with potential exposure to amosite asbestos, glass and rockwool fibres. Groups of workers with at least 5 years' potential exposure were recruited and blood and urine samples were collected for biomarker measurement. In addition, they underwent a series of clinical examinations. Worker exposure to fibres was estimated by direct monitoring of their occupational environment as well as through the use of questionnaires. A large number of biomarkers of inflammation, immunotoxicity, oxidative stress and genotoxicity were examined. In general no significant or consistent pattern of biomarker changes was observed, consistent with the very low levels of fibre exposure of the workers under investigation.

### **Benefits and Beneficiaries:**

#### **Community added value and contribution to EU policies**

By investigating the cellular pathways leading to fibre genotoxicity, FIBRETOX generated scientific data of relevance to the hazard and risk assessment of mineral fibres, thus providing support for European Union regulatory policies in the areas of occupational and environmental health, product safety, as well as for the policy of replacement of asbestos with other fibres.

#### **Contribution to Community social objectives**

The need for strengthening the scientific basis for setting standards to protect human health from the effects of environmental toxicants is well recognised. FIBRETOX results may be utilised for improved setting of limits of exposure to mineral fibres, through improved low-dose extrapolation and risk assessment. This would be valuable not only in the context of occupational exposure and protection of worker health, but also for the health protection for the general population from mineral fibres in general and asbestos in particular. This is because, even after a complete ban of asbestos, asbestos-containing products which are already in use (e.g. asbestos cement-constructed buildings, boards or pipes) will remain in existence for many decades. This means that corresponding risks to the general population or to specific occupational groups (e.g. building maintenance workers, asbestos-abatement workers, etc) will remain and may even increase in the future, as demolition and disposal of asbestos-based materials increases. Consequently, the need for improved understanding, assessment and monitoring of the toxic effects of asbestos, especially at low levels of exposure, remains important.

#### **Exploitation and dissemination**

In addition to the publication of project results in international scientific journals and presentations at national and international congresses, a Workshop on the Genotoxicity and Carcinogenesis of Mineral Fibres, in the context of the 33<sup>rd</sup> Annual Meeting of the European Environmental Mutagen Society (Aberdeen, 24-28 August 2003), was organized for the presentation of the FIBRETOX project. The proceedings of this Workshop were published in a Special Issue of the journal Mutation Research (Mechanisms of Genotoxicity and Carcinogenesis of Mineral Fibres, edited by S.A. Kyrtopoulos, FIBRETOX co-ordinator; Mutation Research, vol. 553, issues 1-2, Pages 1-124, 3 September 2004). Finally, the project's main results were presented at a meeting with fibre industry representatives held in Brussels in May 2004.

