Laura Prandi (1998-2001)
Ph.D. Bio-chemical Sciences

Any kind of particulate retained in the lung is noxious and elicits an inflammatory response from the body defences; some, however, like silica and asbestos, exhibit a specific and severe pathogenicity, well beyond the simple inflammation, which ends up with pneumoconiosis, and in the case of asbestos, bronchogenic carcinoma and malignant mesothelioma.This clearly suggests that toxicity is at least partly related to some chemical properties of the dust. The form of the particle also plays a role, as fibres are much dangerous than isometric particles. The fact that fibres of similar size have different pathogenic potential, however, confirms that both shape and chemical composition have to be considered, when dealing with this kind of pathogenicities.

There is a remarkable difference between the kind of toxicity of most chemicals and the one related to the inhalation of mineral particulate. The latter typically involves the introduction in the body of substances, which are mainly insoluble or poorly soluble. As a consequence the particle will reside a very long time in the body – most diseases do in fact appear only long after the exposure – and reactions mainly occur at the interface between the solid particle and the biological medium (intracellular or extracellular). The surface thus governs most chemical processes (reactivity, adsorption, solubility) relevant to the diseases initiation and progression.

Thus, three major aspects have been considered relevant to lung toxicity caused by inhaled particulate: the form of the particle, its chemical composition and its biopersistence.

Many chapters of this thesis regard asbestos. Asbestos encompasses a diverse family of fibrous silicates, which have been largely used in various manufactures in the past because of their exceptional physico-chemical characteristics. Their use is now banned in most Western countries because of the well-established correlation with the development of cancers (pleural mesothelioma and bronchogenic carcinoma) in populations exposed to airborne asbestos fibres . Asbestos belong to two main groups: amphiboles (chain silicates) and serpentines (sheet silicates). Their fibrogenic and carcinogenic effects are well established but the molecular mechanisms responsible for these adverse biological responses are not yet fully understood . In particular, great attention has been given in the past few years to the crucial role that iron in the fibre may play in generating ROS (Reactive Oxygen Species) in the proximity of target cells. Chrysotile, the main representative of the serpentine group, has the chemical composition and may contain iron in variable amounts up to about 0.7% in weight only as a substitute for magnesium ions in the octahedral (brucite) sheet formed by edge-sharing $MgO_2(OH)_4$ octahedr a. Chrysotile has been used in 90% of the applications of asbestos and is less carcinogenic in humans than crocidolite or amosite. Amosite and crocidolite are the best-known representatives of the amphibole group. Iron ions are constitutive of these silicates, their chemical formulae being and , respectively.

The crystal structure of the amphiboles can be described in terms of a basic structural unit formed by a double tetrahedral chain (corner linked SiO_4 tetrahedra) of composition . These silicate double-chains share oxygen atoms with alternate layers of edge sharing MO_6 octahedra, where M stands for a variety of cations (mostly Mg^{2+}, Ca

$^{2+}$

, Fe
$^{2+}$

or Fe
$^{3+}$

). The total amount of iron in amosite and crocidolite is in the range 27-29% in weight. Amphiboles exhibit prismatic cleavage: when crushed or milled, they fracture along the octahedral layers giving rise to acicular fragments. Individual fibres are usually about 0.2 um in diameter, and they tend to aggregate into bundles.

Many investigations have shown that the biological effects of asbestos were ameliorated in the presence of deferrioxamine, a potent iron chelator, suggesting a crucial role for iron in the overall toxicity. Not all iron in the fibre appears to be involved in pathogenicity, but only some few iron ions in specific active sites at the surface, whose coordination and redox state still are to be identified . Iron may also be mobilised from the fibre by low molecular-weight chelators and attain cellular DNA in this soluble form. Research in our and other laboratories in the past decade, mostly devoted to the evaluation of the generation of ROS and consequent DNA damage, has evidenced four different iron sites in the amphiboles among which the most reactive ones appear to be those where the iron ion is more isolated10, more coordinatively unsaturated and more easily removable from the silicate framework. It has been hypothesised that redox cycling and iron removal and deposition on the fibres might be the mechanisms whereby the active iron sites involved in ROS release may be continuously regenerated in vivo.

In the present thesis work we have therefore investigated the kinetics of ion release in the presence of various chelators and the uptake of iron from a weak iron complex. The mechanism of iron mobilisation, re-deposition and redox cycling at the surface are therefore relevant to long term toxicity and biopersistence of asbestos fibres. A study on the modifications in the chemical composition at the fibre surface following incubation in aqueous solutions of chelators suggested a complex mechanism of ion release. Results obtained with cyclic voltammetry, an electrochemical technique new to the field, as only one pioneering paper is available , concerning the redox state and the mobility of the iron ions located at the octahedral sites in the silicate framework of amphibole asbestos, are also reported. We have considered the most common forms, crocidolite and amosite, which are also the richest in iron (30 % in weight).

Any study aimed at investigating new ways of modifying the asbestos surface, in order to obtain a material where the above adverse features are eliminated, will contribute to establish new and sound procedures for asbestos inactivation and disposal. In this respect, this work investigates the processes associated to some treatments of asbestos in order to modify its physico-chemical properties. We have studied the effects of thermal treatments, performed both in air and in vacuo, and the effects of both iron removal and iron uptake on the reactivity of the surface of asbestos. Considering that the decontamination of asbestos fibres dispersed over wide soil areas and in waters obviously requires a different approach from what performed for asbestos localized in buildings, we thus investigated the ability of some soil fungi to grow in the presence of crocidolite asbestos fibres, and their potential to release iron chelators apt to modify the nature of the surface of the fibres at the atomic level.

Different forms of pulmonary diseases have been described also among workers exposed to

hard metals. Hard metal alloys are made of a mixture of tungsten carbide particles cemented in cobalt metal powder. Epidemiologic and experimental data suggest that patogenicity is not induced by cobalt alone but requires the simoltaneous inhalation of particles of cobalt metal and other compounds such as tungsten carbide. It was proved that this interactive toxicity is mediated by activated oxygen species (AOS) produced by the association of cobalt metal and metallic carbide particles but not by cobalt or tungsten alone . In this thesis work, we go deeper insight the mechanism of generation of free radicals from the mixtures of WC and three different metals: Co, Ni and Fe.