Bice Fubini
Università degli Studi di Torino, Italy
Department of Chemistry IFM and Interdipartimental Center “G.Scansetti” for the Study of Asbestos and other Toxic Particulates

Influence of Physico-Chemical Properties of Asbestos Fibers on their Biological Activity

ASBESTOS MECHANISMS OF TOXICITY WORKSHOP
U.S. Environmental Protection Agency, Chicago, June 12 & 13 2003
The molecular mechanisms of asbestos related diseases are still obscure…

“...no single mechanism fully accounts for all the complex biological abnormalities caused by asbestos.” Kamp&Weitzman, Thorax, 54, 638-652, 1999

Has physical chemistry something to do with it?
past two decades

*from:* toxic minerals considered as a single molecular substance  
*to:* heterogeneous and dynamic state of the surface of a particle or fiber

role played by physico-chemical properties in asbestos toxicity
progressive achievements

the fibers

differences among various asbestos forms and sources

role of crystallinity and chemical composition

pathogenic fibers other from asbestos: e.g. erionite, ceramic fibers, SIC wiskers
the fiber surface

“it is the surface which “dialogates” with cells and tissues”

- surface composition ≠ bulk
- role of surface area and surface composition
- active sites at the surface
- ions may be selectively leached from the fiber surface
- various mechanisms for free radical generation

modifications in vivo

speciation
selective leaching
deposition
paradigms

From the ’60s up to now

fibrous habit, fiber dimensions, aspect ratio

mineral composition, iron generated free radicals, ROS, RNS

durability and biopersistence

each failed to explain all experimental data on the assumption that one single physico-chemical characteristic should account for pathogenicity
with “particulate toxicants”

- **mechanisms**
  - multiple fiber/living matter interactions implied in the adverse response

- **physico-chemical aspects**
  - heterogeneity in surface reactive sites

**pathogenicity**

not one single feature but a set of features, acting at different levels
inhale fibres/living matter interactions

reaction with extracellular matter

direct action on target cells (damage/proliferation)

cell death (necrosis/apoptosis)

reactions between cell and fiber products

macrophage activation: release of oxidants, cytokines, growth factors; recruitment of AM and PMN

successful phagocytosis

clearance

damage to target cells (epithelial/mesothelial)
physico chemical properties relevant to health effects

the complex network in fiber/cell interactions

from mechanisms to possible inactivation routes

further developments and research needs
physico chemical properties relevant to health effects

- fibrous habit
- potential to generate free radicals
- the complex role of iron
- reactivity towards endogenous antioxidants
- hydrophilicity and protein adsorption
fibrous habit

deposition, clearance
migration alveoli $\rightarrow$ interstitium $\rightarrow$ pleura
frustrated phagocitosis

release of oxidants, lytic enzymes, cytokines

depth of macrophage

*Induction of mesotheliomas*

• mainly fibrous materials (*asbestos, erionite, ceramic fibers*)
• long thin fibers $>>$ short fibers
fibrous habit

asbestos type

*fiber dimensions, role of shape*

amphiboles >> serpentine

health effects

mesothelioma > lung cancer, asbestosis
generation of free radicals

in vivo & in vitro

asbestos generate free radicals

asbestos stimulate cells → ROS, RNS

iron implicated in various biological responses to fibers
Relevant books & reviews


most studied asbestos

Amosite
Crocidolite
Chrysotile

ROS and RNS evidenced

$\text{HO}^\cdot, \text{H}_2\text{O}_2, \text{O}_2^-, \text{NO}$

evidence of the role of ROS in DNA damage

$\text{RTE (rabbit tracheal epithelium)}$
$\text{Rabbit pleural mesothelial cells}$
$\text{WI-26, A549 (human alveolar epithelial cells)}$

evidence of the role of Fe in ROS generation and damage
first direct evidence of radicals generated by asbestos fibers

Weitzman and Graceffa, 1984

contribution to the understanding of the chemical role played by asbestos …

Aust, Kamp, Gulumian, Pézerat, Rahman, Vallyathan, Weitzman & their associates
Men + target molecule → free radical

Me^{n+} active surface site

spin trapping technique (EPR)

- nature of free radical released
- abundance
- kinetics

surface modifications → variations in free radical release → hypothesis on the active surface site
In the presence of $\text{H}_2\text{O}_2$

Fenton reaction

$$\text{Fe(II)}_{\text{(surface)}} + \text{H}_2\text{O}_2 \rightleftharpoons \text{Fe(III)} + \text{OH}^- + \cdot\text{OH}$$

confined to compartments where $\text{H}_2\text{O}_2$ is present
In the absence of $\text{H}_2\text{O}_2$

**Haber –Weiss Cycle**

Reductant + Fe(III)\_\text{surface} \rightleftharpoons \text{oxidized reductant} + \text{Fe (II)}

\[
\text{Fe (II)} + \text{O}_2 \rightleftharpoons \text{Fe (III)} + \text{O}_2 \cdot -
\]

\[
\text{O}_2 \cdot - + 2\text{H}^+ + \text{e}^- \rightleftharpoons \text{H}_2\text{O}_2
\]

\[
\text{Fe(II)} + \text{H}_2\text{O}_2 \rightleftharpoons \text{Fe(III)} + \text{OH}^- + \cdot\text{OH}
\]

**Cleavage of a carbon-hydrogen bond**

\[
\text{-C—H} + \text{X} \text{\_surface site or free radical} \rightleftharpoons \text{-C} \cdot + \text{HX}
\]
fiber generated free radicals

**effects of redox thermal treatments on crocidolite**

Fenton-like reaction

- Original sample
- Outgassed in vacuum at 400°C
- Heated in air at 400°C

**cleavage of C-H bond**

- Original sample
- Outgassed in vacuum at 400°C
- Heated in air at 400°C

**Vacuum: reduction**

**Air: oxidation**

**OH · yield unaffected, C-H cleavage related to the presence of Fe(II)**

*Fenoglio et al., Redox Rep. 6, 235-241 (2001).*
Ascorbic acid restores free radical activity on crocidolite.
Fenton activity
- insensitive to redox state
- requires surface iron
- catalytic surface site

H-C cleavage
- only if Fe (II) is present
- blunted by surface oxidation
- active site re-generated by endogenous reductants (ascorbate, GSH)

Consistent with prolonged effects in vivo
at which stage do free radicals play a role?

- fibre uptake enhanced by ROS
- complement activation
- fiber derived free radicals/ROS
- ROS, RNS from AM, PMN
- peroxynitrite, $\text{ONOO}^- \cdot$ from cell and particle derived NO and $\text{O}_2^- \cdot$

damage to target cells
asbestos type

variability among asbestos type and sources of the same mineral

health effects

open questions

chemical characteristics of the free radical generating sites
the complex role of iron

which iron is active

how to evidence active iron

modification of iron in the fiber *in vivo*
**iron in asbestos**

\[ \text{Fe}^{2+} \text{ is present at least in traces, also in chrysotile and tremolite asbestos substituting for } \text{Mg}^{2+} \text{ ions} \]

**serpentines**
- Chrysotile
  \[ \text{Mg}_6\text{Si}_4\text{O}_{10}(\text{OH})_8 \]

**amphiboles**
- Crocidolite
  \[ \text{Na}_2(\text{Fe}^{3+})_2(\text{Fe}^{2+}, \text{Mg})_3\text{Si}_8\text{O}_{22}(\text{OH})_2 \]
- Amosite
  \[ (\text{Fe}^{2+},\text{Mg})_7\text{Si}_8\text{O}_{22}(\text{OH})_2 \]
- Anthophyllite
  \[ (\text{Mg},\text{Fe}^{+2})_7\text{Si}_8\text{O}_{22}(\text{OH})_2 \]
- Tremolite
  \[ \text{Ca}_2\text{Mg}_5\text{Si}_8\text{O}_{22}(\text{OH})_2 \]
- Actinolite
  \[ \text{Ca}_2(\text{Mg},\text{Fe}^{+2})_5\text{Si}_8\text{O}_{22}(\text{OH})_2 \]

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**the complex role of iron**
the complex role of iron

active iron

in the fibre (only a small fraction, brought at the surface, sitting on particular surface sites)

mobilized bound to low molecular weight chelators 

Aust & associates

deposited from environment endogenously

Aust & associates, Ghio & associates
the complex role of iron

kinetics of iron mobilized from crocidolite by various chelators

iron removal depends upon the chemical nature of the chelator
but is unrelated to the stability constant of the complex

endogenous

employed as drugs

esogenous

nmol Fe/mg of solid

t (days)

citrate

ascorbate
deferoxamine

dex

EDTA

Ferrozine

L. Prandi et al. submitted
total iron content of the fiber does not parallel:

- iron mobilized by chelators
- iron activity in free radical generation

active sites: few, well dispersed at the surface poorly coordinated ions
iron ions with different free coordination valencies may be evidenced by means of adsorption of NO

FT-IR spectroscopy
at which stage does iron play a role?

- Fiber uptake
- Chelators assisted iron release
- Chelated iron may attain DNA
- Surface iron may catalyze free radical generation
- Iron or iron derived radicals may react with macrophage oxidants
  \[
  \text{NO}^\cdot + \text{O}_2^- \rightarrow \text{ONOO}^\cdot
  \]
- Clearance
- Damage to target cells (epithelial/mesothelial)
the complex role of iron

asbestos type

variability depends upon not only iron content but also upon crystallochemical structure

health effects all?
In vitro reaction with endogenous antioxidants

- Ascorbate consumption
- Cysteine consumption

Oxidation of ascorbate

Glutathione depletion

Ascorbate → Dehydroascorbate

Dehydroascorbate reductase

GSH → GSSG

GSSG reductase
reaction with endogenous antioxidants

effect of ascorbate on crocidolite asbestos: iron removal

incubation for 25 days in a 10 mM ascorbate solution (changed each 5 days)
The surface is progressively deprived of NO adsorbing sites.

Short time incubation: the most uncoordinated iron ions are removed first.

Long time incubation: the structure anchoring Fe ions is eroded.

New highly uncoordinated sites are exposed.

asbestos fibers appears to act also at this level

much attention given to ROS/RNS generation

reaction with endogenous antioxidants

asbestos type

cysteine & ascorbate consumption

amosite > crocidolite > chrysotile > tremolite

health effects all?
Adsorption of proteins

protein adsorption on asbestos fibers can alter their interaction with target cells

e.g.

IgG and macrophages stimulation

adsorption of proteins

vitronectin increased fiber internalization by rabbit pleural mesothelial cells


vitronectin coating increased intracellular oxidation and apoptosis of mesothelial cells

adsorption of proteins

ferritin on amosite fibers induces exposure of protein iron core and enhances free radical release

Otero-Arean et al., Res. Chem. Intermediates, 25, 177-185, 1999
asbestos type

protein adsorption

great variability among various asbestos due to surface charge and functionalities
the complex network of fiber cells interactions

- Short vs long fibers
- Fiber generated ROS
- ROS and RNS from cells
- Depletion of antioxidants at the fiber surface
- Protein adsorption
association between surface properties and in vivo responses

Protein adsorption
Ascorbate/GSH depletion
Iron release

Iron at active site
Free radicals

Iron bound to chelators

Long fibers
Active sites for Fenton reaction and other ROS generation
Protein adsorption

Short fibres
Smooth surface
Hydrophobicity
Protein adsorption

Clearance

Damage to target cells
all theories taking into account just one chemical feature failed...

not a single physico chemical property but a set of features imparts pathogenicity to a given dust

specific distribution of the active surface sites

subsequent interactions of the fiber with cells and tissues

intensity of the adverse reactions
from mechanisms to possible inactivation routes

Once assessed which physico-chemical features are involved in the pathogenic mechanisms...

Try to modify some of those features aiming to reduce or blunt the biological activity of the fibers
Tentative routes for the inactivation or containment of asbestos fibres in different environmental circumstances, i.e. manufactures, removal, dispersed on soils

**iron deposition**

**thermal treatments/solid state reactions**

**mechanical treatments**
*short, comminuted vs long fibers*

**surface coating**
*spontaneous polymerization*

**chelator assisted iron depletion**
*selective leaching, bioremediation*
iron deposition

Treatment with ferric iron salts

- Reduced ability for ROS generation and Iron mobilization
  Gulumian and associates, 1989-1993

Iron covered ceramic fibres

- Attenuated cytostatic, cytotoxic and transforming potency on Syrian embryo cells
thermal treatments/solid state reactions

Very high temperatures (e.g. plasma ashing)

Asbestos are fully destroyed

How to obtain transformation of active surface sites in milder conditions?
**effect of heating crocidolite on ROS generation**

<table>
<thead>
<tr>
<th>Fenton reaction</th>
<th>cleavage of C-H bond</th>
<th>cleavage of C-H bond in the presence of ascorbate</th>
</tr>
</thead>
<tbody>
<tr>
<td>· OH from $\text{H}_2\text{O}_2$</td>
<td><img src="#" alt="Original" /></td>
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</tbody>
</table>

*Only above 800°, i.e when the silica framework starts disrupting, the fibers loose their potential to generate free radicals*

*Tomatis et al., *Langmuir*, 18, 4345-4350, 2002*
mechanical treatments (short, comminuted vs long fibers)

long and short amosite fibers

(short obtained by prolonged milling, effects measured at equal mass)

in vivo and in vitro tests

long >> short


Revisited recently by the Torino group
(Riganti et al. submitted, Tomatis et al., to be published)

- Differences in most relevant surface properties
- Long but not short ones inhibit antioxidant defenses and cause cell damage

Does prolonged milling inactivate the fibers?
Eugenol, a natural compound extracted from cloves, spontaneously polymerizes into lignin-like products at the fiber surface of crocidolite asbestos.

The reaction takes place in the presence of hydrogen peroxide.

Fenoglio et al., Chemm. Com., 21, 2182-2183, 2001
polymerization is catalyzed by the fiber surface itself

*proposed molecular mechanism*

formation of lignin–like products, with radical blunting activity

*exploits the Fenton activity of the fiber*
before

after polymerization

crocidolite

crocidolite treated with eugenol

crocidolite treated with eugenol

10 µm

100 µm

300 µm

10 µm

before polymerization
...after polymerization

Fenton activity inhibited

DMPO-OH•

Fenoglio et al., Chem Comm, 21, 2182-2183, 2001

Iron mobilized by desferrioxamine

Dramatically reduced iron mobilisation

Turci et al., to be published
chelator assisted iron release / a possible bioremediation route

effect on the fibre of prolonged incubation with chelators

bioremediation with soil fungi?
Kinetics of iron release by deferoxamine.

Iron release by deferoxamine much exceeds surface iron.

Prandi et al., to be published
After iron removal also silicon is present in the supernatant.

**TEM**

*before*

*after treatment with deferoxamine*

Following iron diffusion, the silica framework collapses.
How long may the process proceed?

What about microorganisms releasing chelators?
growth of fungi in the presence of asbestos

*Oidiodendron maius* Zn

**intimate interaction between fungal hyphae and fibers**

Iron released is comparable to what mobilized by desferrioxamine

the potential of the fibres to generate free radicals is fully suppressed

Fenton-like reaction
\[ \cdot \text{OH from } \text{H}_2\text{O}_2 \]

EPR spectra of [DMPO-OH°]

Prolonged growth of iron extracting fungi on the fibres yields transformations at the fiber surface similar to those taking place after incubation in desferrioxamine
asbestos stimulates mnsod production

Mn-SOD antibody recognises specifically one band in the basic area of the gel
iron release in the presence of *F. Oxysporum* from different asbestos fibres

*F. Oxysporum* induces the removal of substantial amounts of iron from all the samples considered

Daghino et al. to be published
further developments and research needs

Impact of surface modifications on the biological responses elicited

proceed in the association of the variety of cellular responses to defined physico-chemical properties

How to validate inactivation of asbestos

appropriate set of chemical, biochemical and cellular tests

How to predict the toxicity of other asbestiform minerals

from chemistry to pathology and no more from epidemiology up the ladder to mechanisms
New fibers: the case of Biancavilla (CT, Sicily, Italy)

23 cases of pleural mesothelioma, up to 2001, with no professional exposure to asbestos

Tremolite- Actinolite / Fluoroedenite

Paoletti et al., Arch. Envir. Health, 55, 392, 2000
Balangeroite from the Balangero mine (Piedmont, I)

View of the former mine, now a lake

$\text{M}_{21}\text{O}_3(\text{OH})_{20}(\text{Si}_4\text{O}_{12})_2$

where $\text{M}$ is $\text{Mg}$, $\text{Fe(II)}$ and $\text{Fe(III)}$
traces of $\text{Mn}$, $\text{Al}$, $\text{Ca}$, $\text{Cr}$, $\text{Ti}$

Compagnoni et al., Am. Min., 68, 1983

Natural rock fragment with balangeroite fibers

Long fibers of balangeroite

Balangeroite structure (110 face)
ACKNOWLEDGMENTS

The experimental work performed in the University of Torino was supported by Regione Piemonte and is the product of the collaborative work of the following people:

Dept. of Chemistry
Raffaella Ceschino
Ivana Fenoglio
Gianmario Martra
Maura Tomatis
Francesco Turci

Dept. of Biochemistry
Elisabetta Aldieri
Loredana Bergandi
Amalia Bosia
Elena Gazzano
Dario Ghigo
Chiara Riganti

Dept. of Plant Biology
Paola Bonfante
Stefania Daghino
Elena Martino
Silvia Perotto

Dept. of Mineralogy:
Elena Belluso, Roberto Compagnoni, Elisa Fornero, Chiara Groppo