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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?

role played by physico-chemical properties in asbestos toxicity

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

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 $\neq$ 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

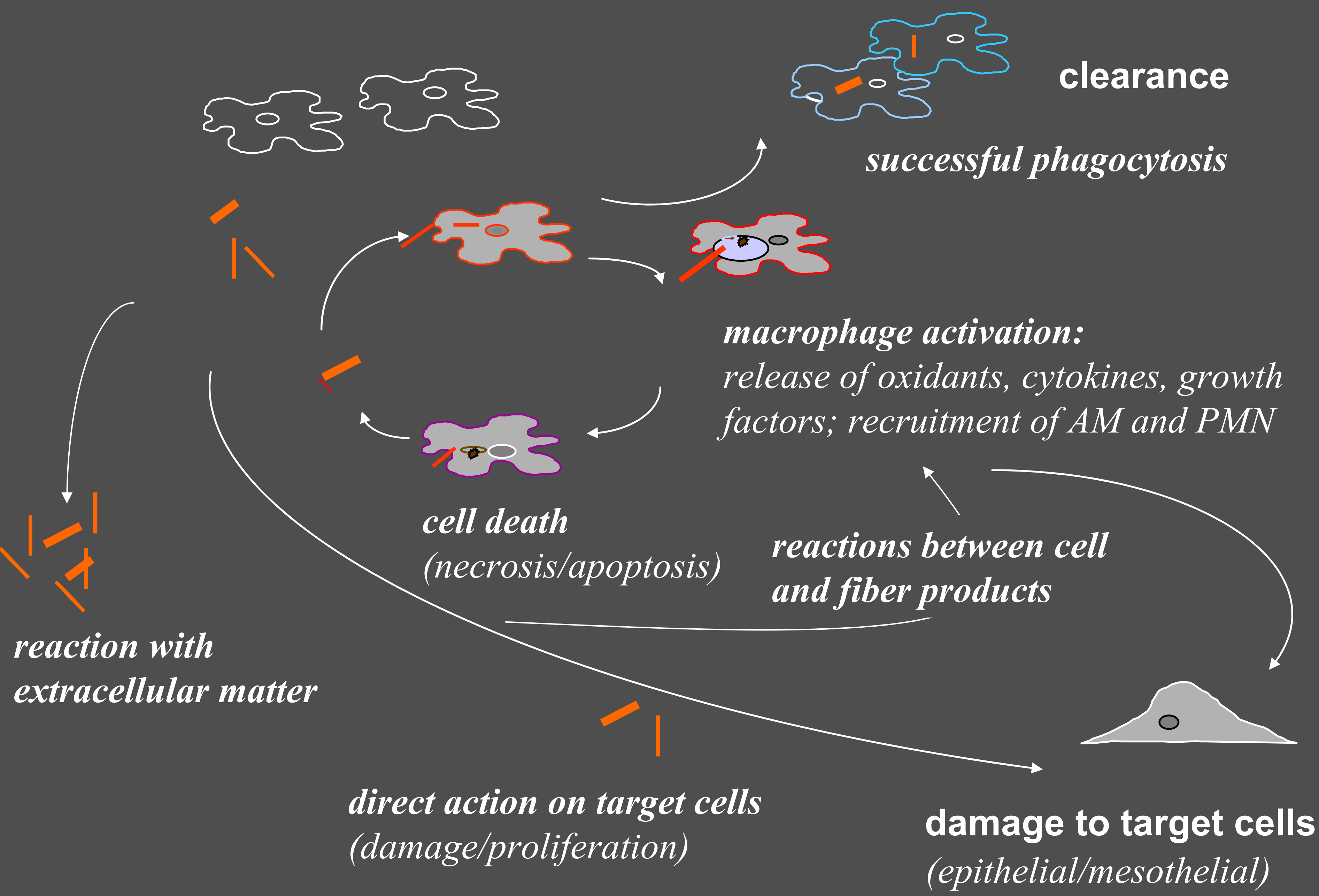
physico-chemical aspects

heterogeneity in surface reactive sites

not one single feature but a set of features, acting at different levels

→ pathogenicity

inhaled fibres/living matter interactions



outline

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

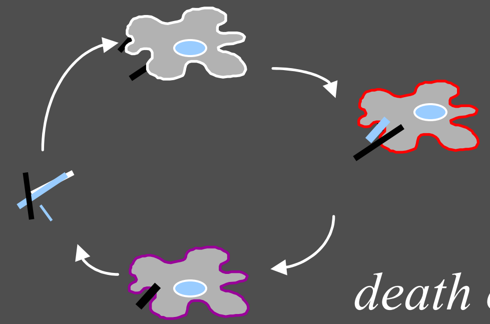
fibrous habit

deposition, clearance

migration alveoli ⇨ interstitium ⇨ pleura

frustrated phagocitosis

release of oxidants, lytic enzymes,cytokines

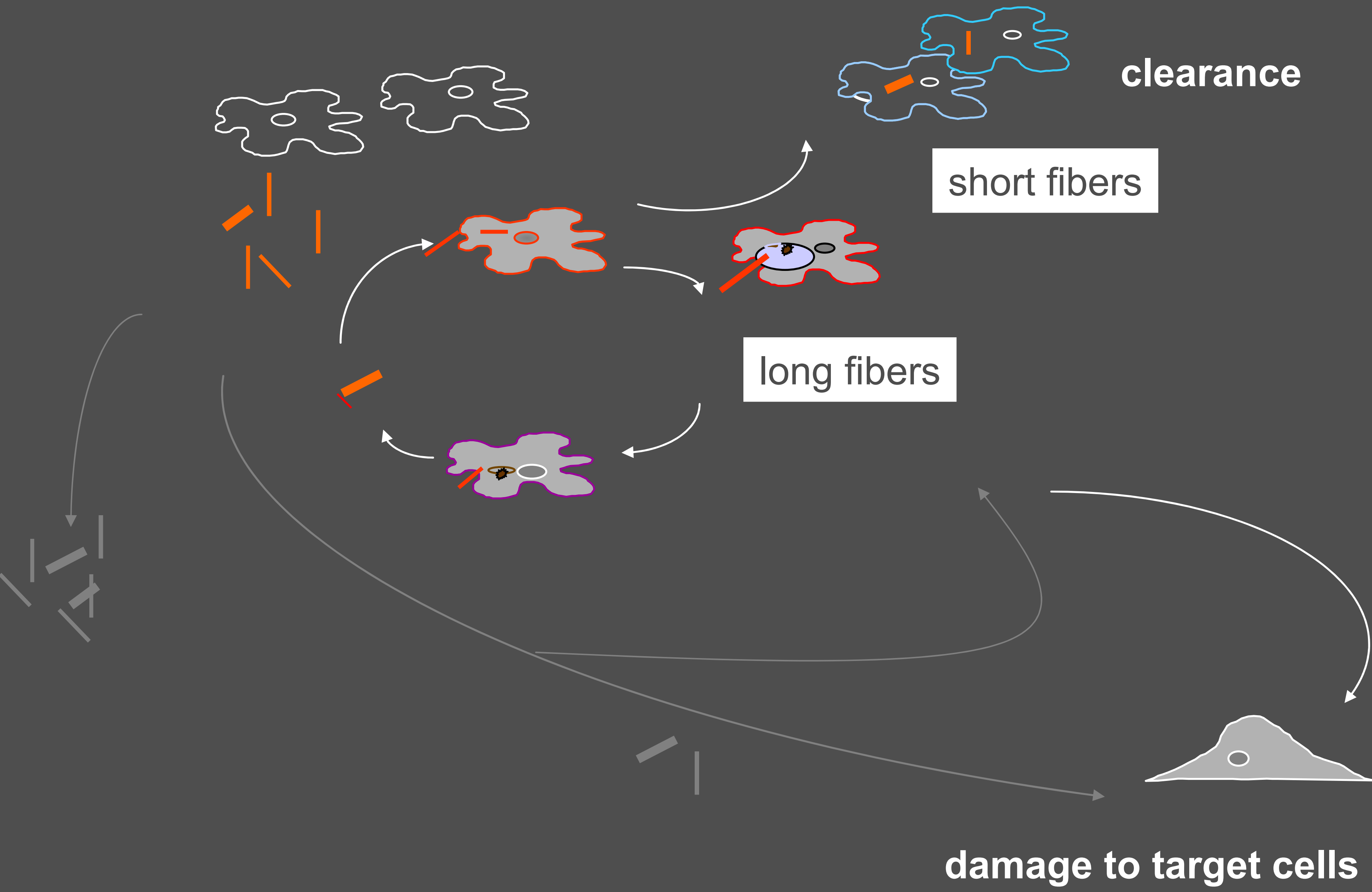


death of macrophage

Induction of mesotheliomas

- mainly fibrous materials (*asbestos erionite, ceramic fibers*…)
- long thin fibers >> short fibers

fate of long vs 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

Mechanisms of Fibre Carcinogenesis*; Kane, A.B., Boffetta, P., Saracci, R.; Wilburn, J.D., Eds.; IARC Scientific Publication No. 140; International Agency for Research on Cancer: Lyon, France,1996; p 11.*

Hardy J.A. and Aust A.E. Iron in asbestos chemistry and carcinogenicity. *Chem. Rev. 95:97-118, 1995.*

*Mossman B.T. and Churg A.*Mechanisms in the pathogenesis of asbestosis and silicosis *Am J Respir Crit Care Med*, *157(5): 1666-80, 1998*

Kamp D.W. and Weitzman S.A. The molecular basis of asbestos induced lung injury. *Thorax, 54:638-652, 1999*

*Kinnula V.L.*Oxidant and antioxidant mechanisms of lung diseases caused by asbestos fibers. *Eur. Respir. J. , 14:706-716, 1999*

Robledo R. and Mossman B. Cellular and molecular mechanisms of asbestos-induced fibrosis. *J. Cell. Phys. 180:158-166, 1999*

Shukla A., Gulumian M., Hei T.K., Kamp D.W., Rahman Q., Mossman B.T. Multiple role of oxidants in the pathogenesis of asbestos-induced fibrosis *Free rad. Biol. Med., 34(9):1117-29, 2003*

most studied asbestos ⇒ Amosite

Crocidolite

Chrysotile

ROS and RNS evidenced ⇒ HO·, H_2O_2 , $O_2^{\cdot -}$, NO

evidence of the role of ROS in DNA damage ⇒ *Rat alveolar macrophages and various cell lines*

evidence of the role of Fe in ROS generation and damage ⇒ *RTE (rabbit tracheal epithelium)*

Rabbit pleural mesothelial cells

WI-26, A549(human alveolar epithelial cells)

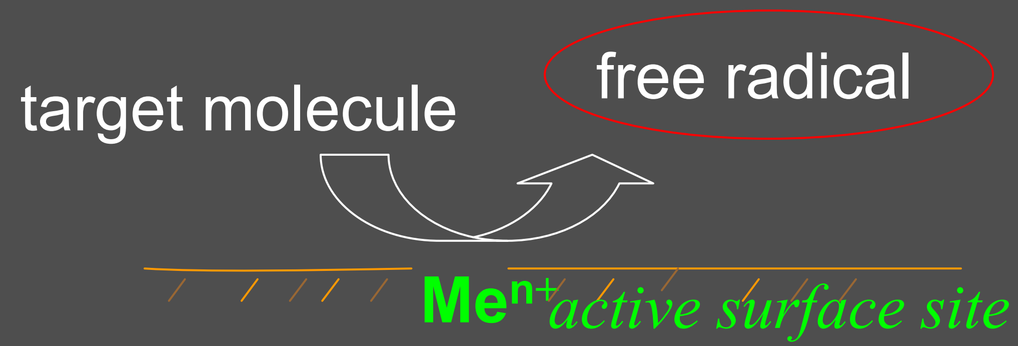
fiber generated free radicals

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



spin trapping technique (EPR) ⇒

- nature of free radical released
- abundance
- kinetics

surface modifications ⇒ variations in free radical release ⇒ **hypothesis on the active surface site**

different mechanisms

In the presence of H_2O_2

Fenton reaction

$Fe(II)_{(surface)} + H_2O_2 \Rightarrow Fe(III) + OH^- + {}^\bullet OH$

confined to compartments where H_2O_2 is present

In the absence of H_2O_2

Haber –Weiss Cycle

Reductant + $Fe(III)_{(surface)}$ ⇨ oxidized reductant + Fe (II)

$Fe(II) + O_2$ ⇨ $Fe(III) + O_2^{\bullet -}$

$O_2^{\bullet -} + 2H^+ + e^-$ ⇨ H_2O_2

$Fe(II) + H_2O_2$ ⇨ $Fe(III) + OH^- + {}^{\bullet}OH$

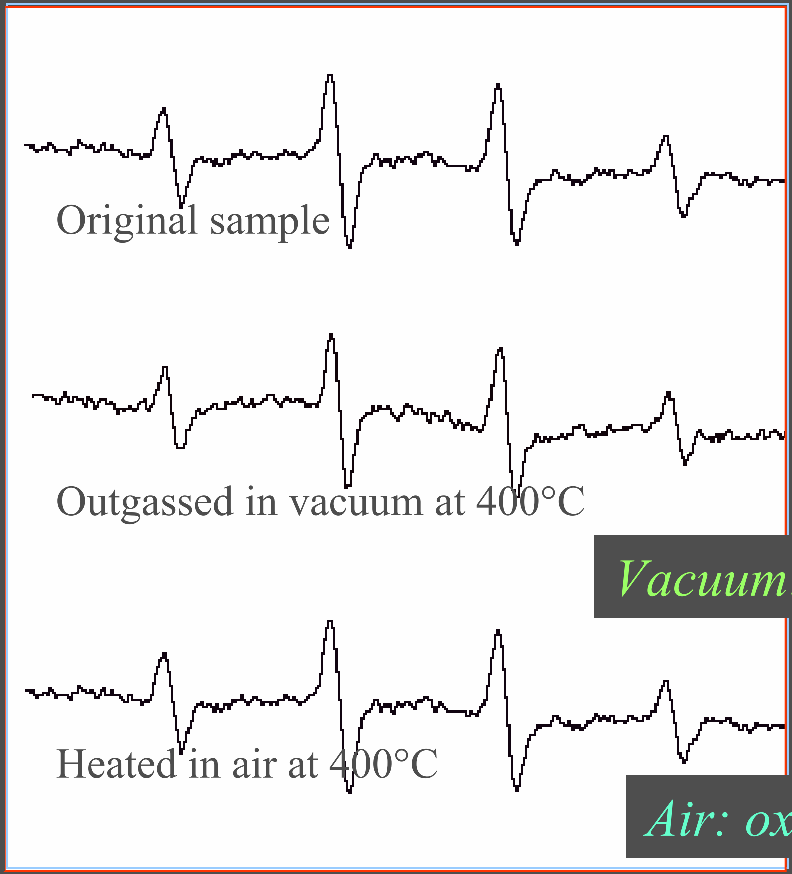
may take place in various biological compartments

Cleavage of a carbon-hydrogen bond

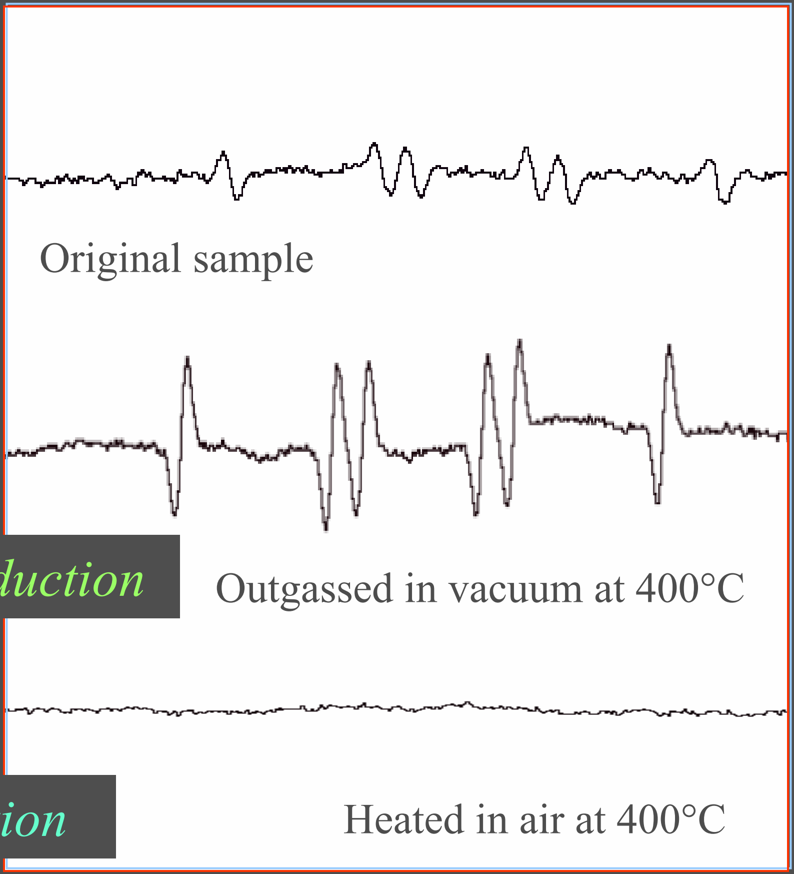
-C—H + $X_{(surface\ site\ or\ free\ radical)}$ ⇨ - C• + HX

effects of redox thermal treatments on crocidolite

Fenton-like reaction



cleavage of C-H bond



Vacuum: reduction

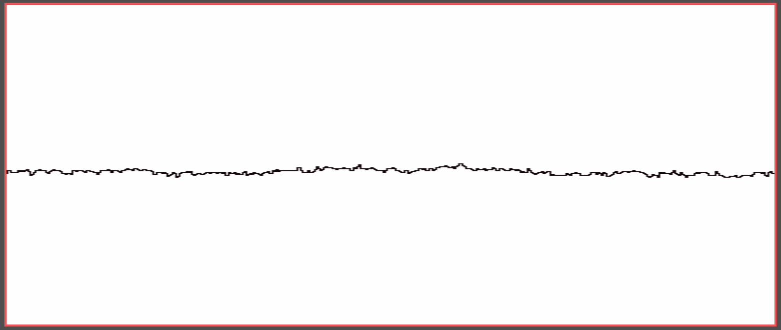
Air: oxidation

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

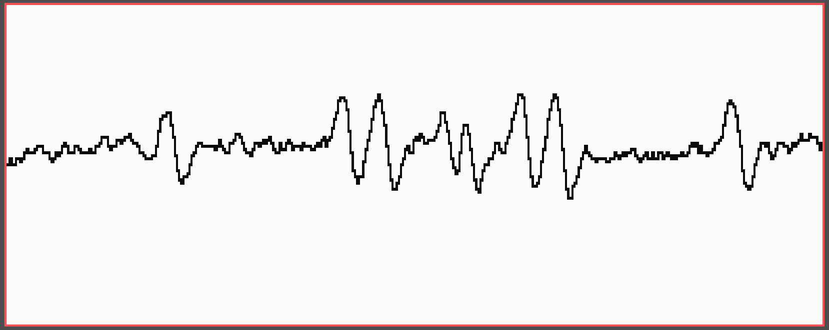
Fenoglio et al., Redox Rep. 6, 235-241 (2001).

→ re-generation of active sites

thermally inactivated crocidolite
(heated in air at 400°c)



in the presence of ascorbic acid
1.5 mM



Ascorbic acid restores free radical activity on crocidolite

different mechanisms

Fenton activity

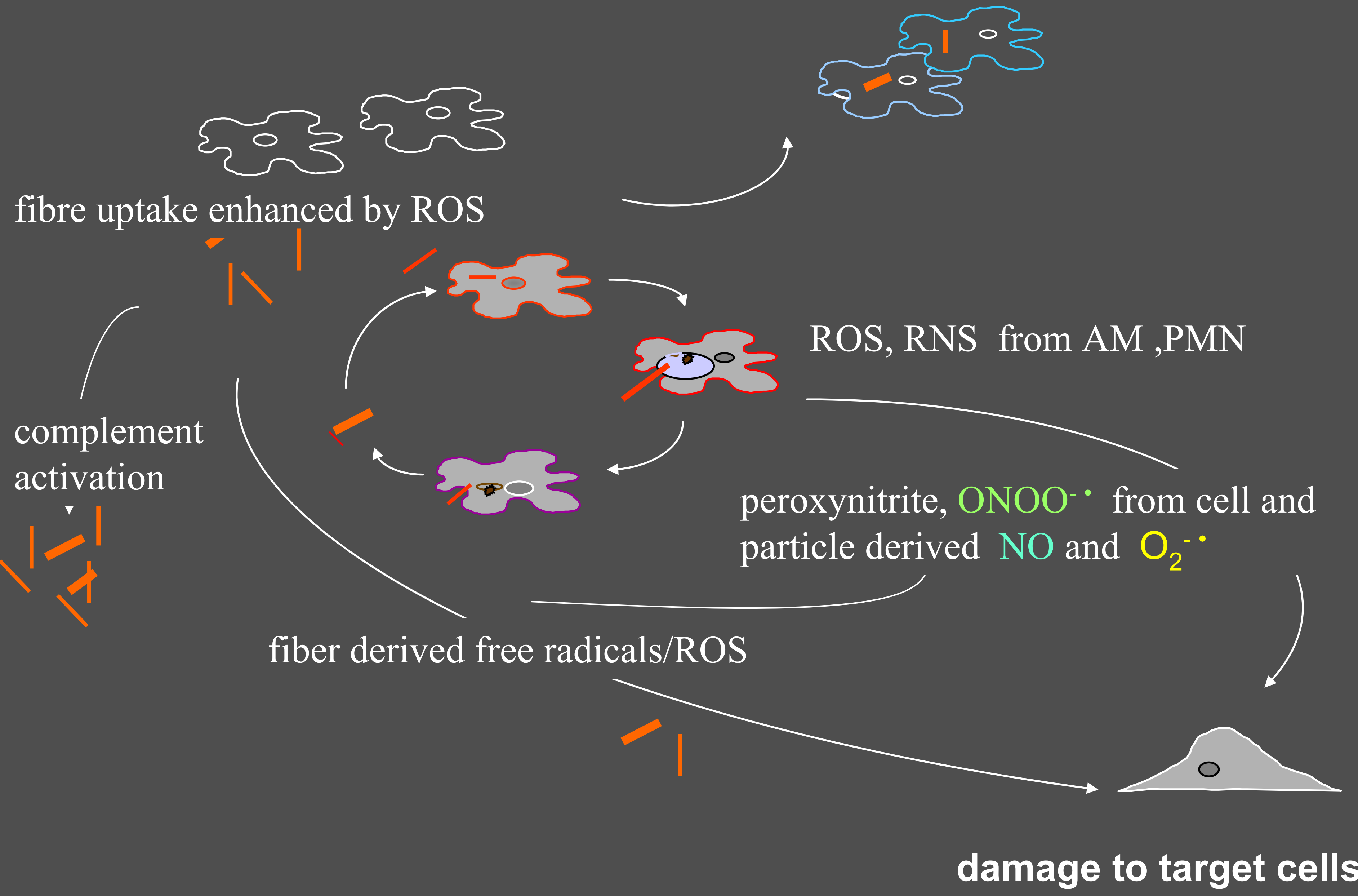
- insensitive to redox state
- requires surface iron
- catalytic surface site

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?



asbestos type

variability among asbestos type and sources of the same mineral

health effects

all ?

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

serpentines

Chrysotile
$Mg_6Si_4O_{10}(OH)_8$

amphiboles

Crocidolite
$Na_2(Fe^{3+})_2(Fe^{2+}, Mg)_3Si_8O_{22}(OH)_2$

Amosite
$(Fe^{+2},Mg)_7Si_8O_{22}(OH)_2$

Anthophyllite
$(Mg,Fe^{+2})_7Si_8O_{22}(OH)_2$

Tremolite
$Ca_2Mg_5Si_8O_{22}(OH)_2$

Actinolite
$Ca_2(Mg,Fe^{+2})_5Si_8O_{22}(OH)_2$

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

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*

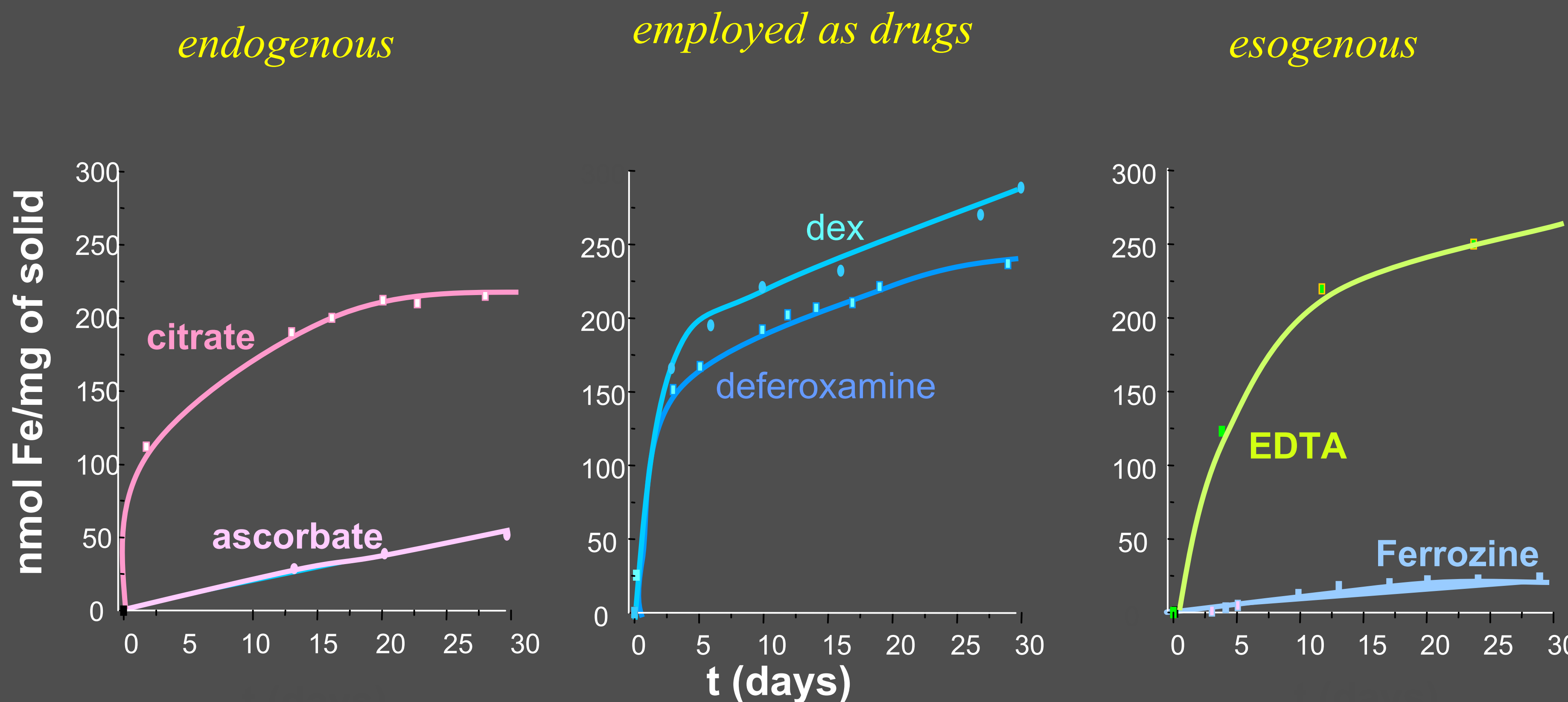
Aust & associates

deposited *from environment*

endogenously

Aust & associates, Ghio & associates

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

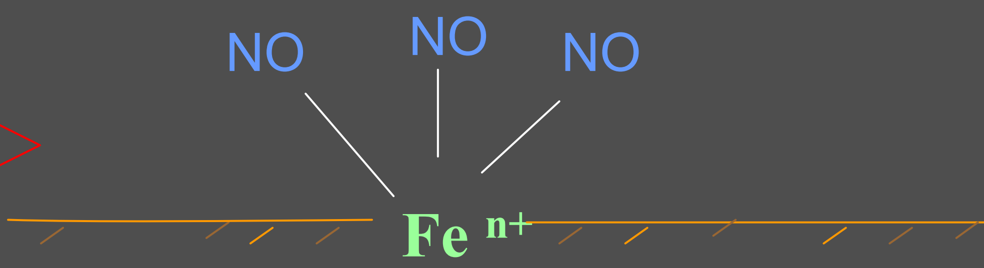
L. Prandi et al. submitted

the complex role of iron

total iron content of the fiber does not parallel:

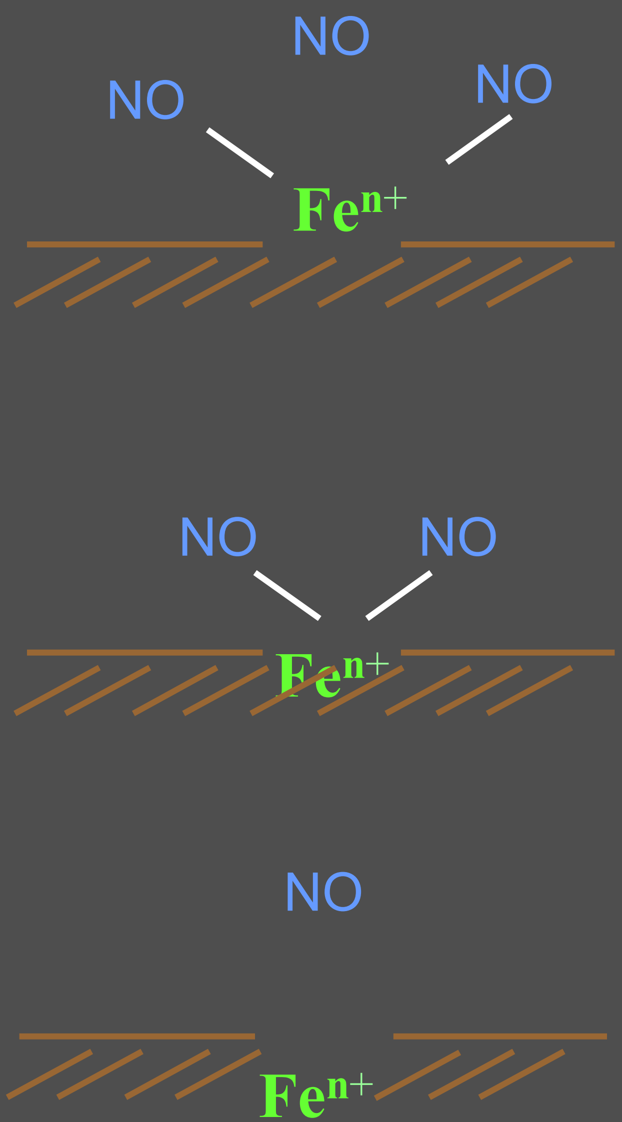
- iron mobilized by chelators
- iron activity in free radical generation

active sites: *few, well dispersed at the surface poorly coordinated ions*

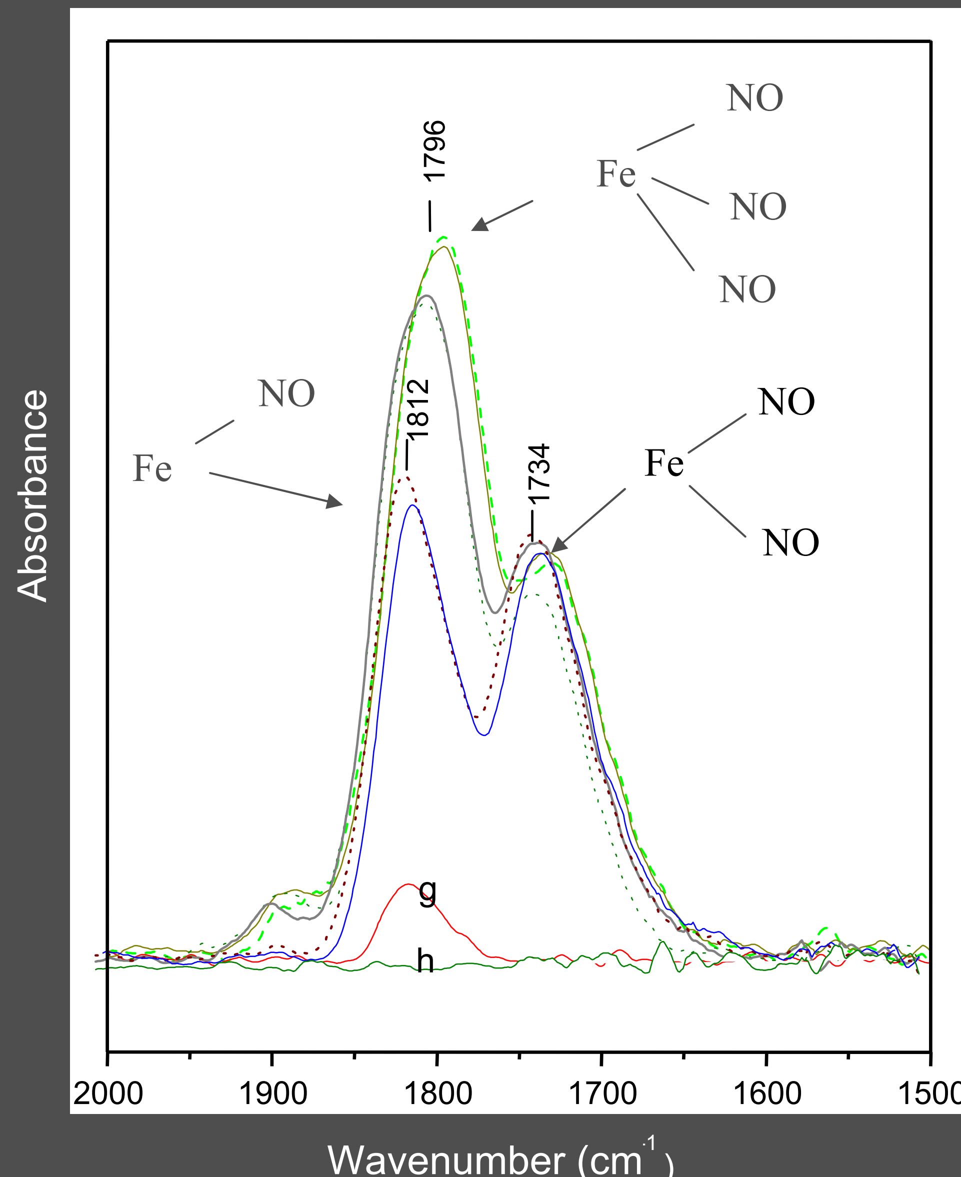


the complex role of iron

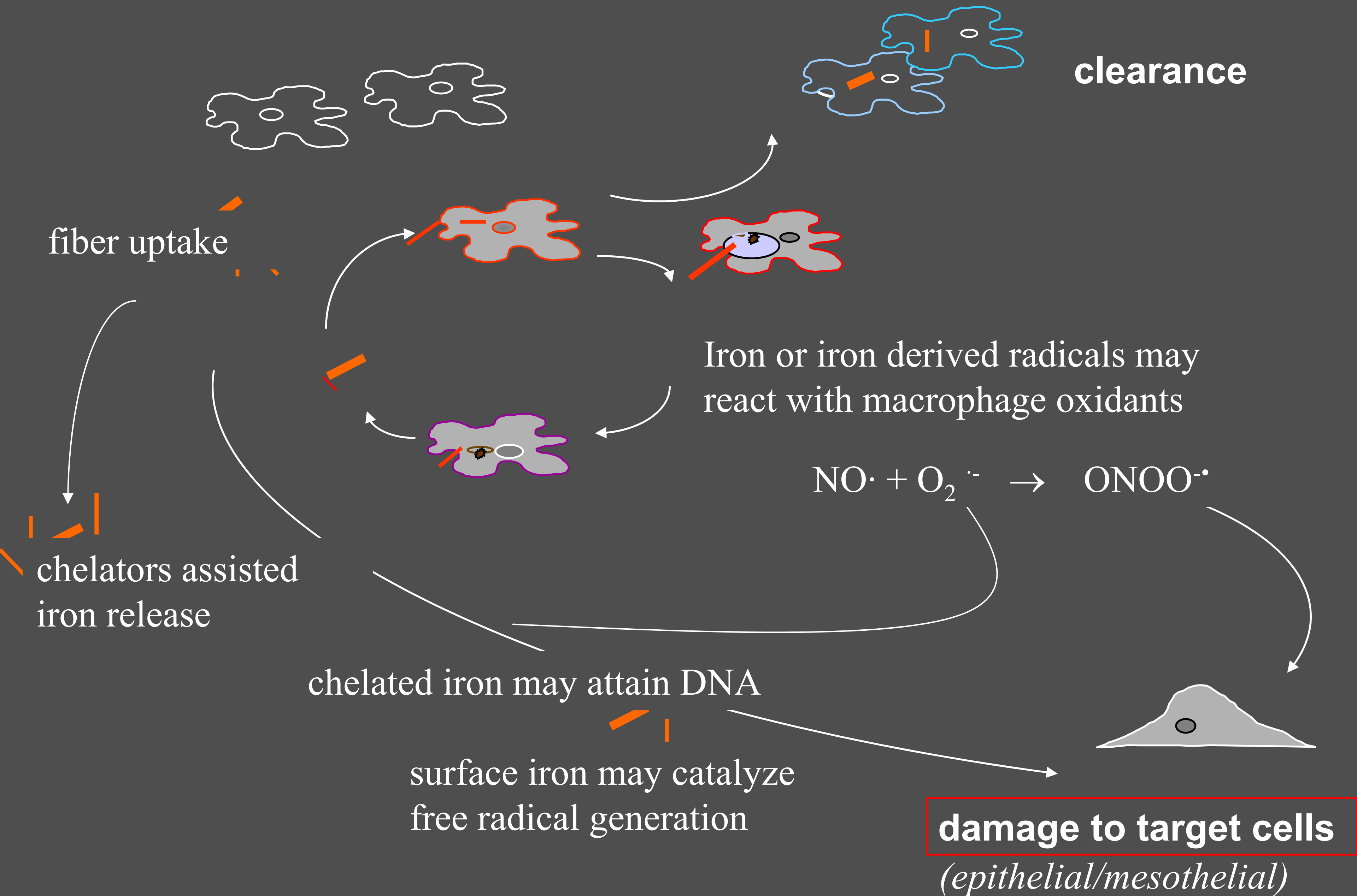
iron ions with different free coordination valencies may be evidenced by means of adsorption of NO



FT-IR spectroscopy



at which stage does iron play a role?



asbestos type

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

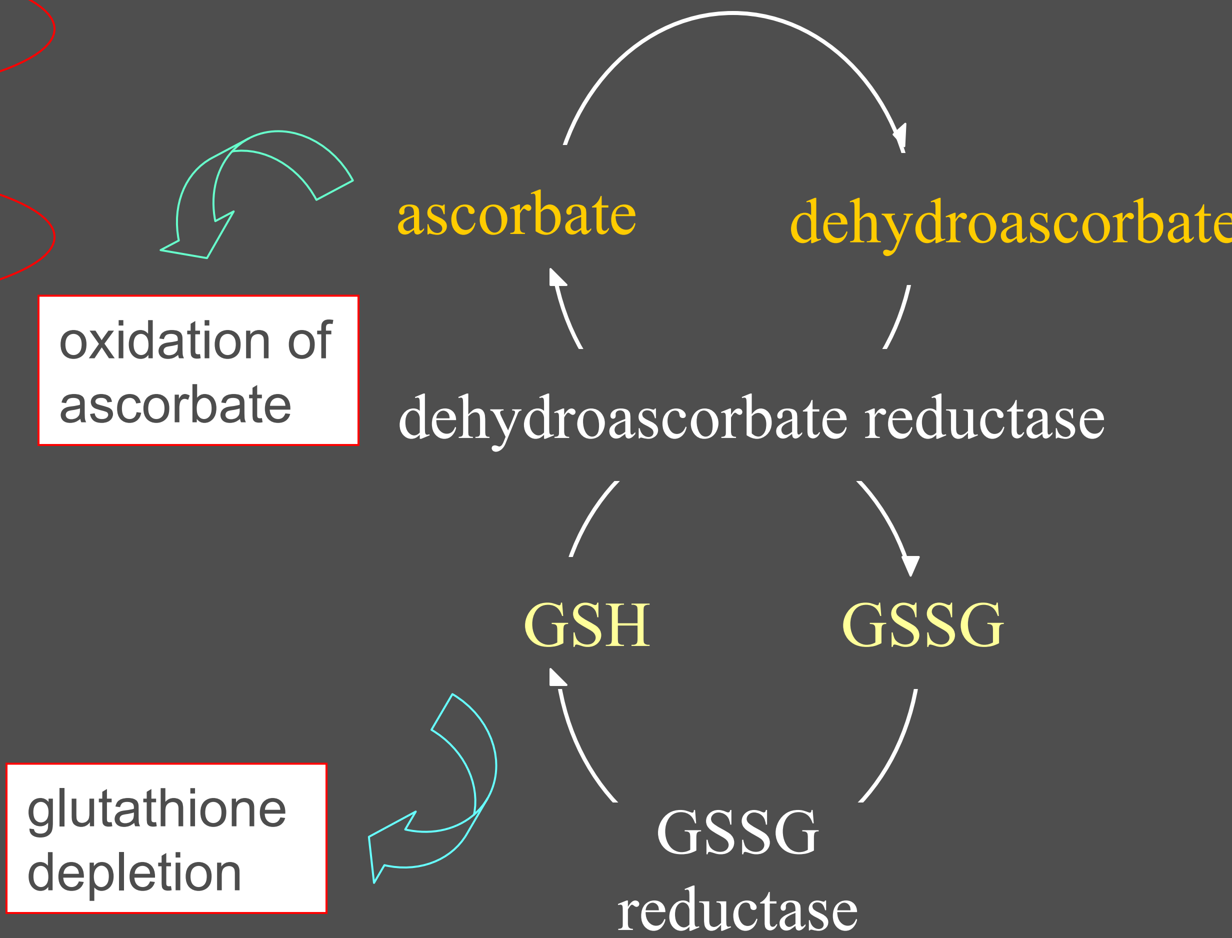
health effects

all?

In vitro reaction with endogenous antioxidants

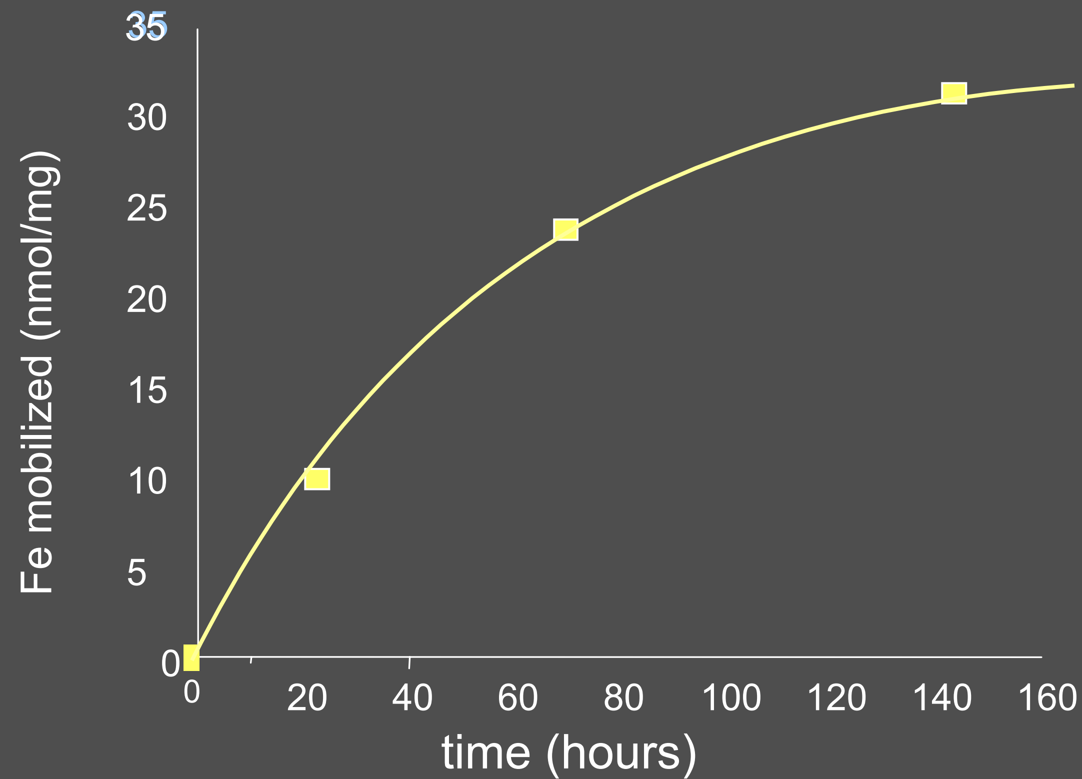
ascorbate consumption

cysteine consumption



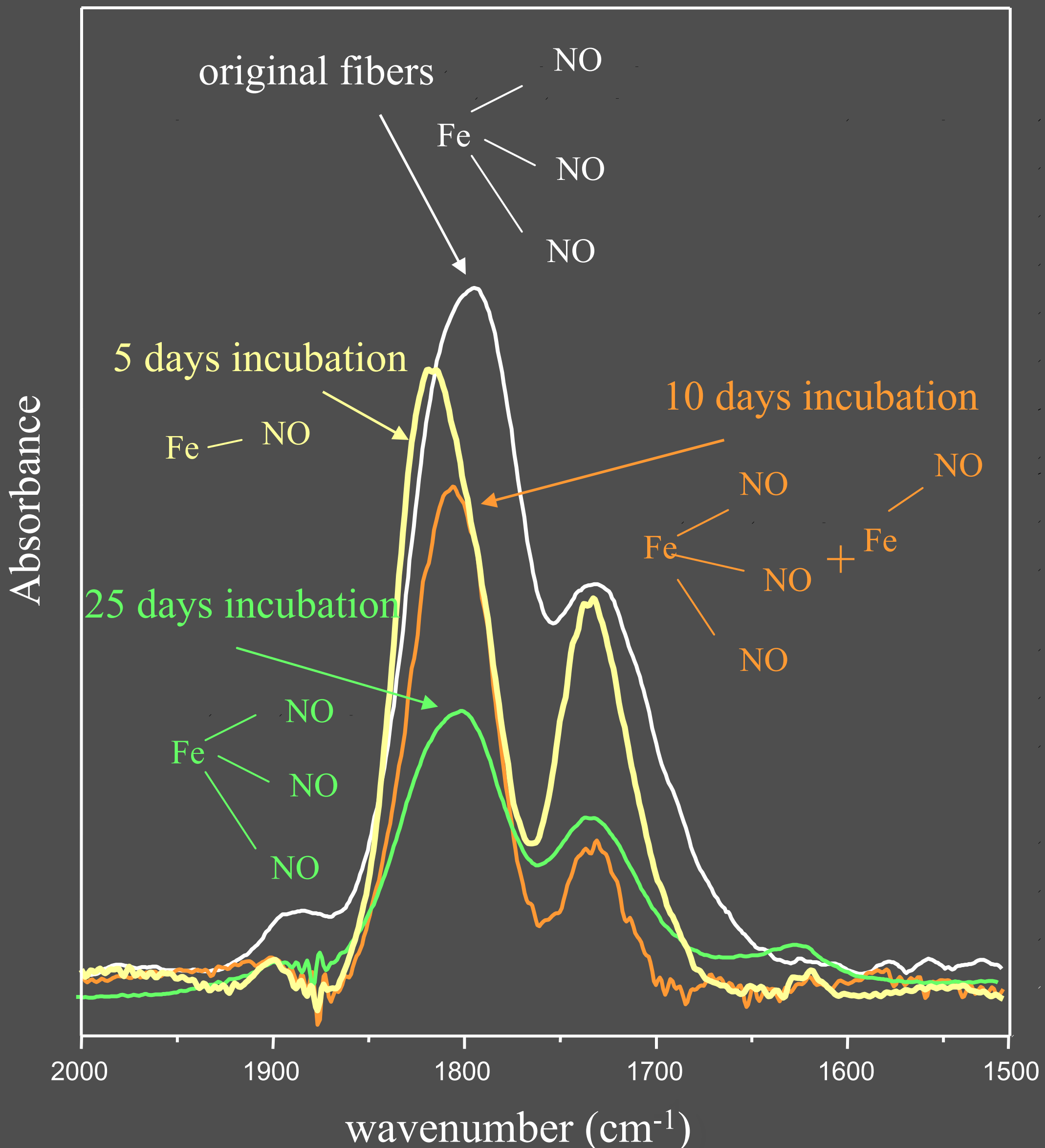
reaction with endogenous antioxidants

effect of ascorbate on crocidolite asbestos: iron removal



incubation for 25 days in a 10 mM ascorbate solution (changed each 5 days)

reaction with endogenous antioxidants



NO uptake (IR spectra of adsorbed NO)

the surface is progressively deprived of NO adsorbing **sites**

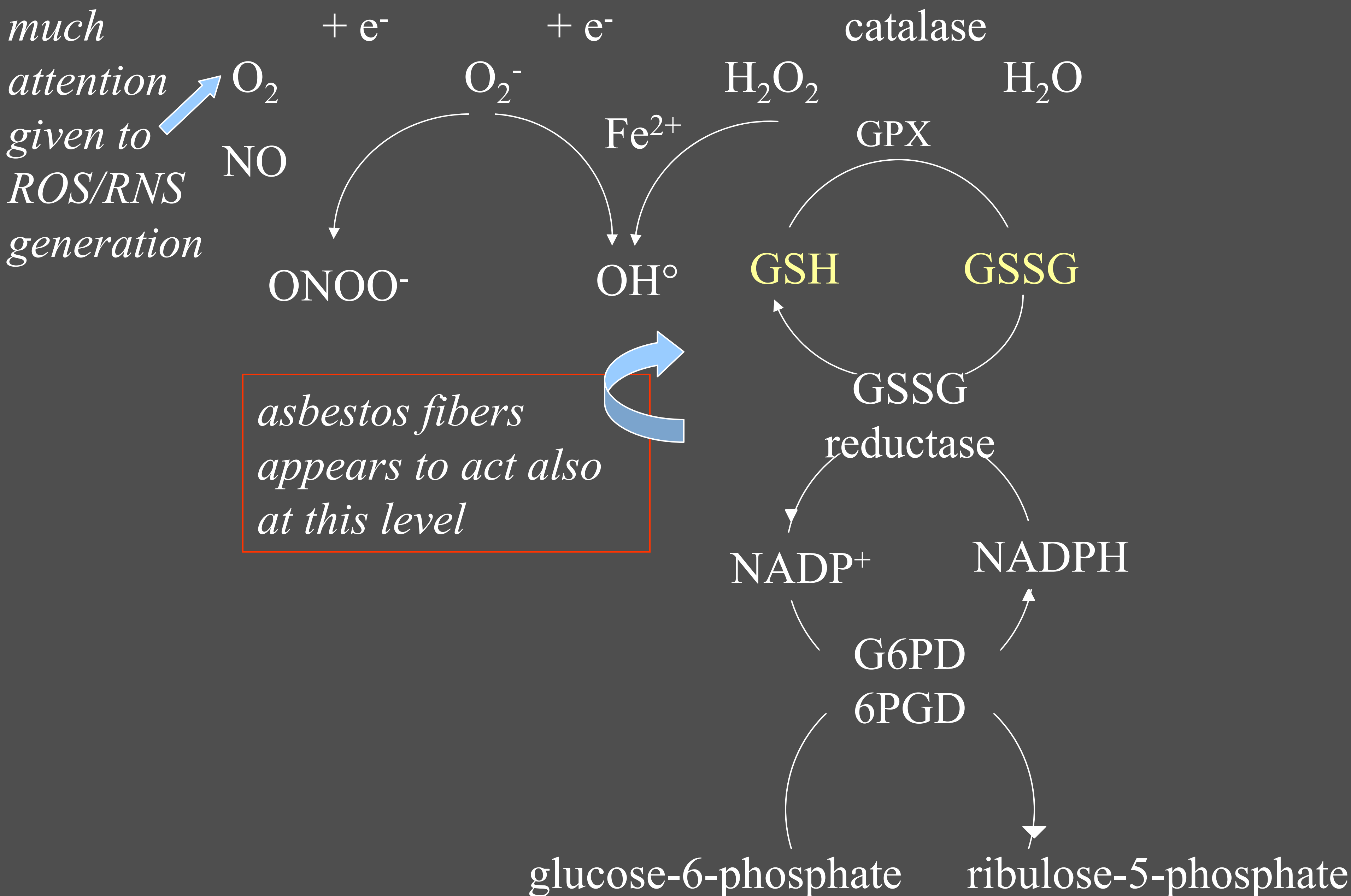
short time incubation: the most uncoordinated iron ions are removed first

long time incubation: the structure anchoring Fe ions is eroded

↓

new highly uncoordinated sites are exposed

Martra et al., Chem. Res. Toxicol. 16, 328-335, 2003.



Riganti et al. Free Rad. Mol. Biol.. ***32****, 938-949, 2002*.

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

Donaldson et al., Exp. Toxicol. Pathol. 47(4):229-31, 1995

Scheule R.K . and Holian A.., Am. J. Respir. Cell. Mol. Bio.; 1(4):313-8 1989 *adsorption of proteins*

vitronectin increased fiber internalization by rabbit pleural mesothelial cells

Boylan et al., J. Clin. Invest. 96(4):1987-2001, 1995

vitronectin coating increased intracellular oxidation and apoptosis of mesothelial cells

Wuet al., Am. J. Physiol. Lung. Cell. Mol. Physiol. 279(5):916-23, 2000 *adsorption of proteins*

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

Fubini et al., J. Toxicol. Environ. Health 52, 101-110, 1997

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

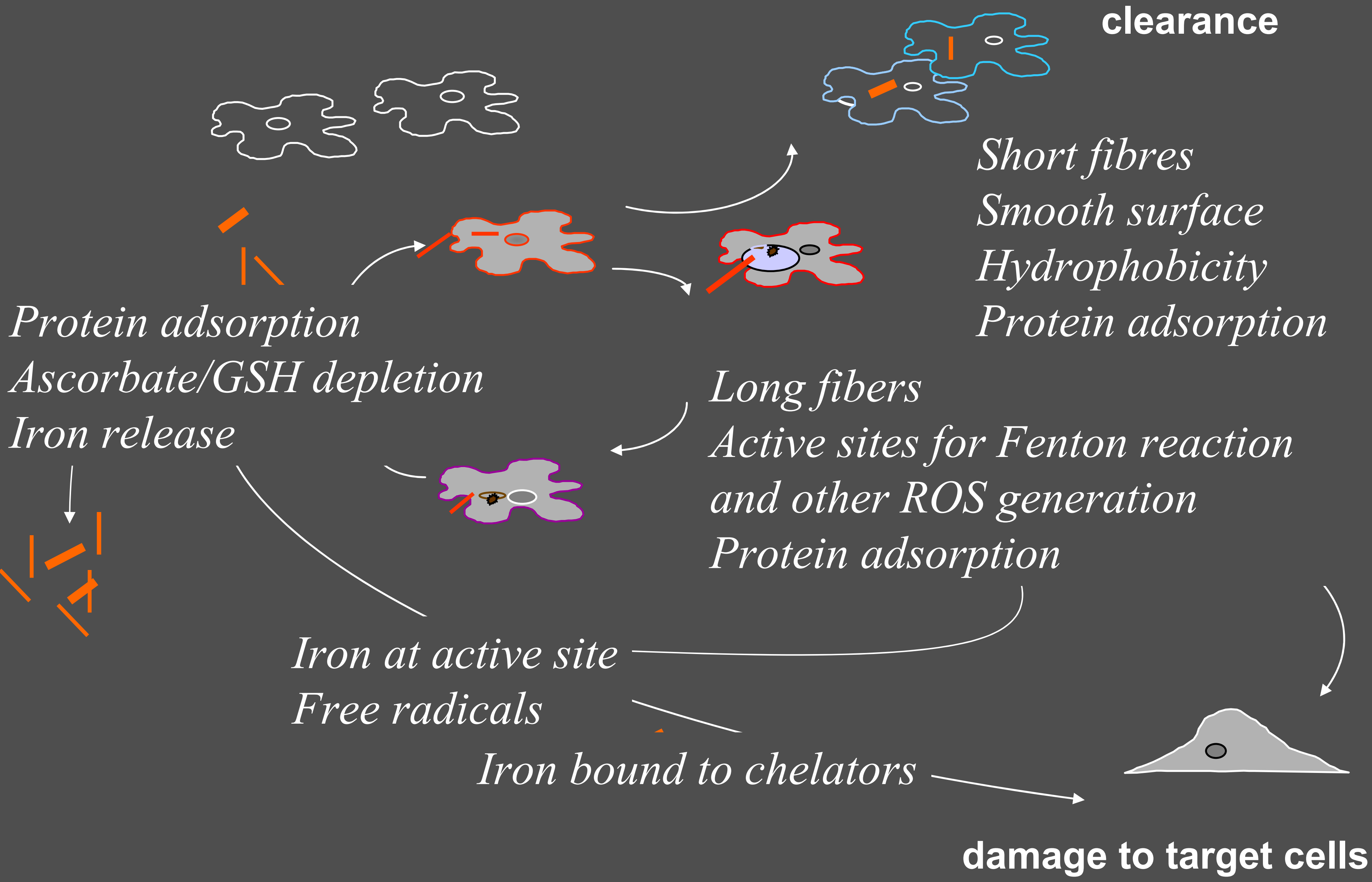
Fiber generated ROS

ROS and RNS from cells

Depletion of antioxidants at the fiber surface

Protein adsorption

association between surface properties and in vivo responses



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. manufacts, 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*

Elias et al., Ann. Occup. Hyg. 46 (l), 176-180, 2002

thermal treatments/solid state reactions

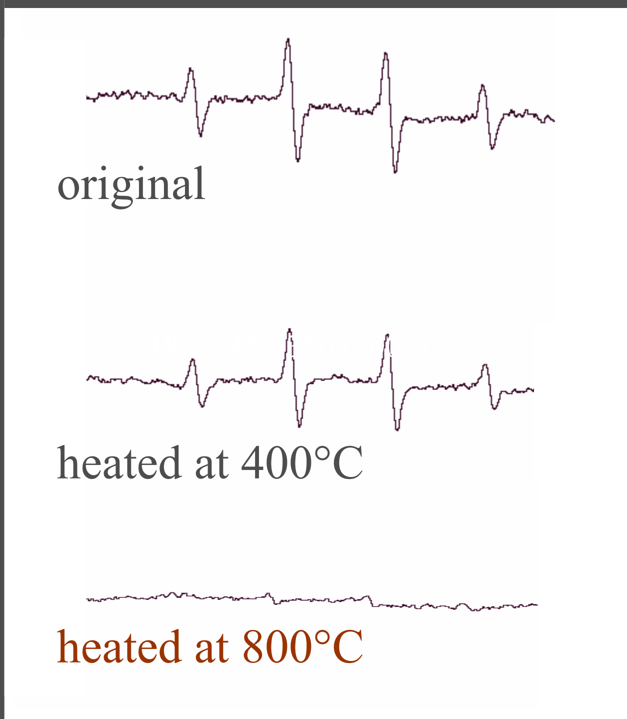
Very high temperatures (e.g. plasma ashing)

⮑ Asbestos are fully destroyed

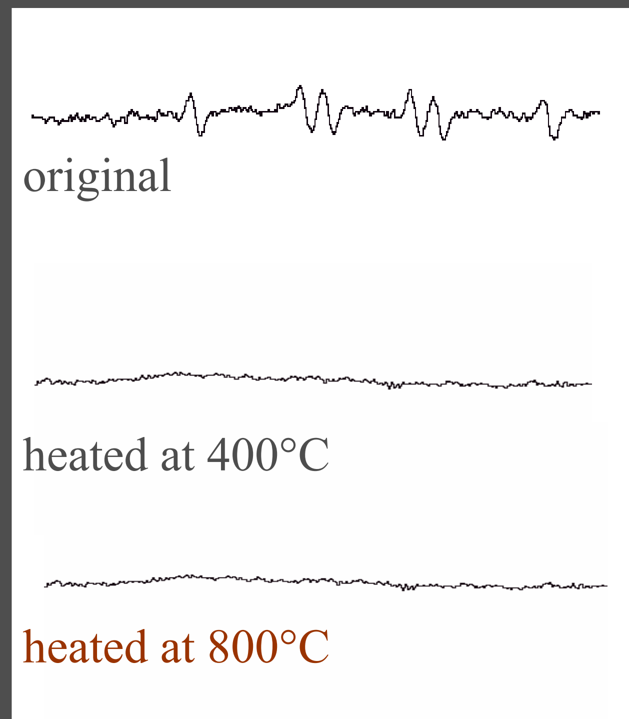
How to obtain transformation of active surface sites in milder conditions?

effect of heating crocidolite on ROS generation

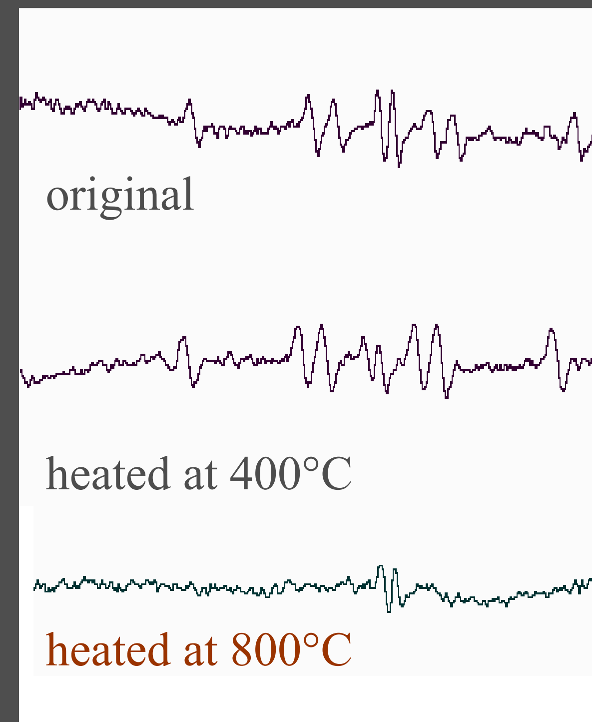
Fenton reaction
· OH from H_2O_2



cleavage of C-H bond



cleavage of C-H bond in the presence of ascorbate



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*

Davis, Donaldson and associates,1985-2000.

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?

surface coating

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

exploits the Fenton activity of the fiber

formation of lignin –like products, with radical blunting activity *before*

after polymerization

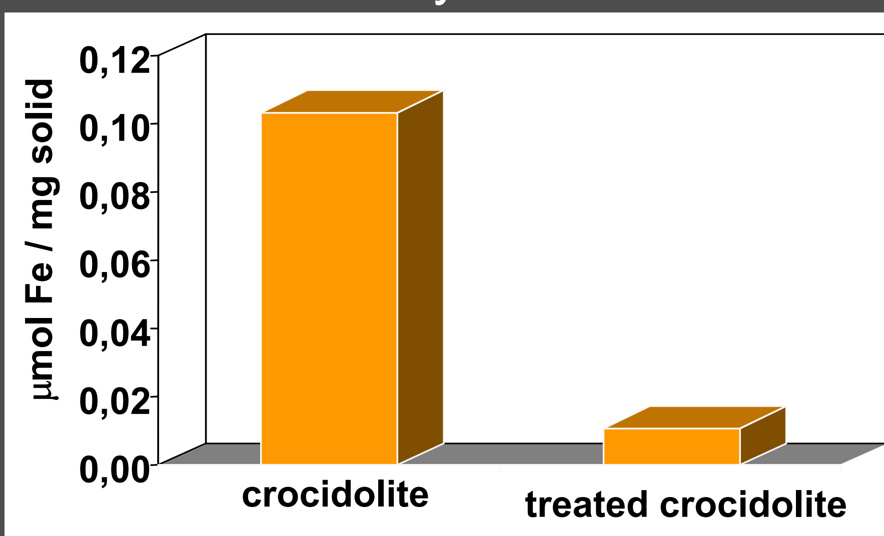
…after polymerization

DMPO-OH•

Fenton activity inhibited

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

Iron mobilized by desferrioxamine



Dramatically reduced iron mobilisation

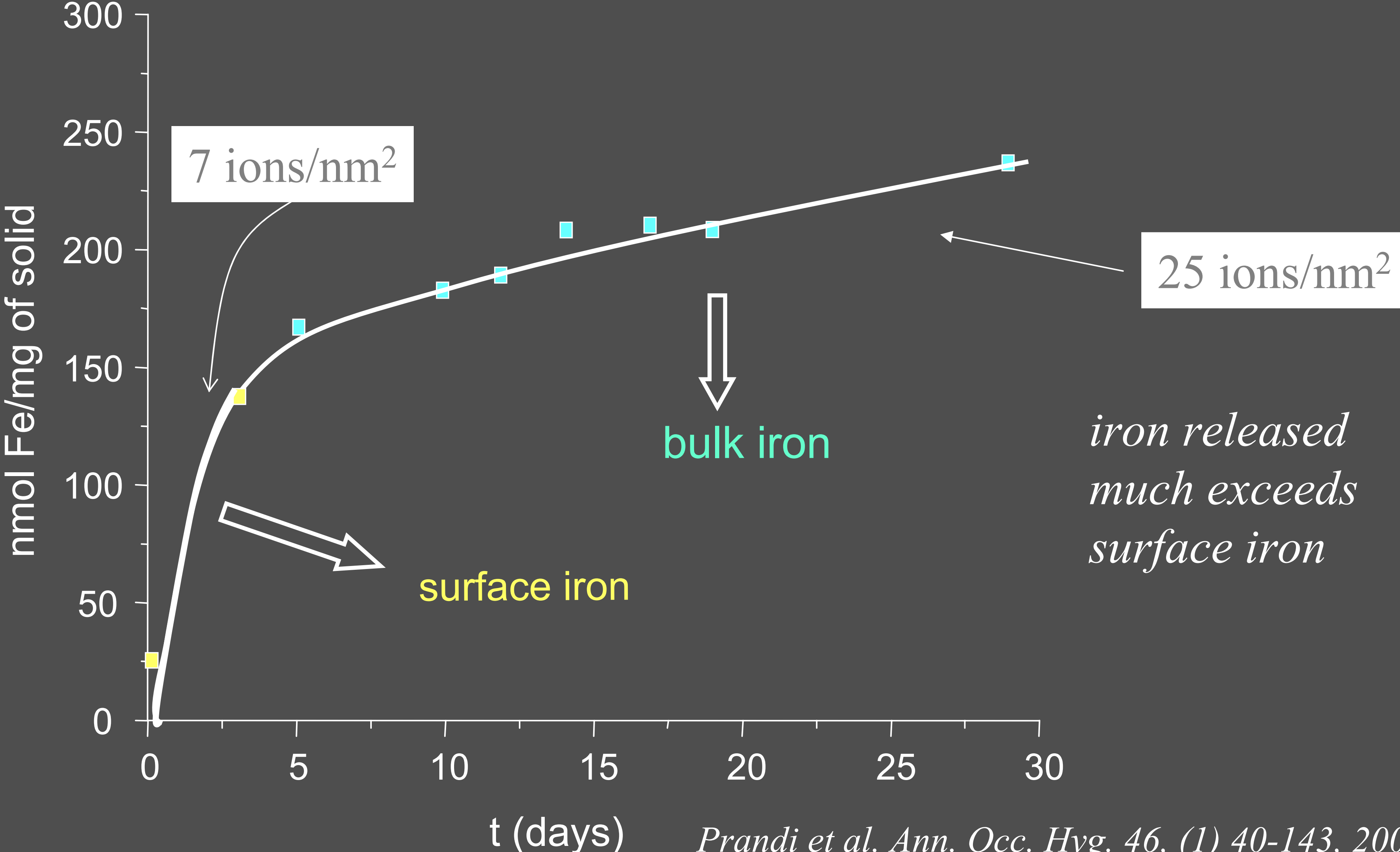
Turci et al., to be published

chelator assisted iron release / a possible bioremediation route

effect on the fibre of prolonged incubation with chelators

bioremediation with soil fungi?

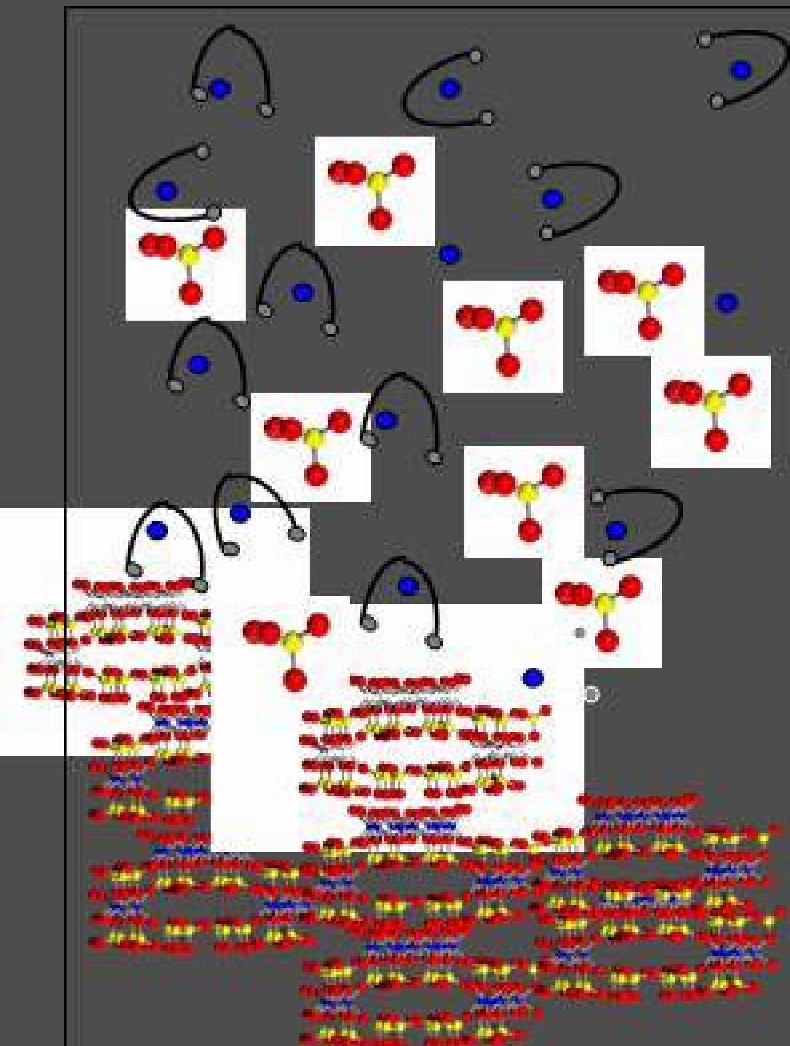
kinetics of iron release by deferoxamine



Prandi et al. Ann. Occ. Hyg. 46, (1) 40-143, 2002
Prandi et al., to be published

overall mechanism

After iron removal also silicon is present in the supernatant.



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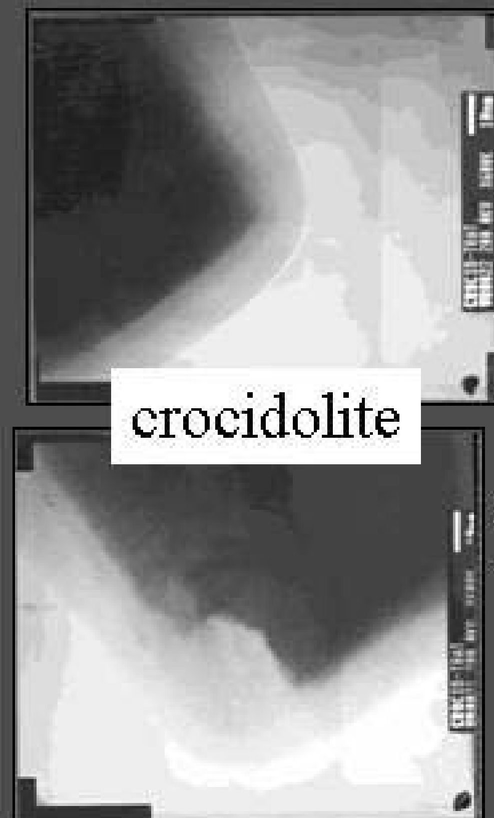


before

after treatment with deferoxamine

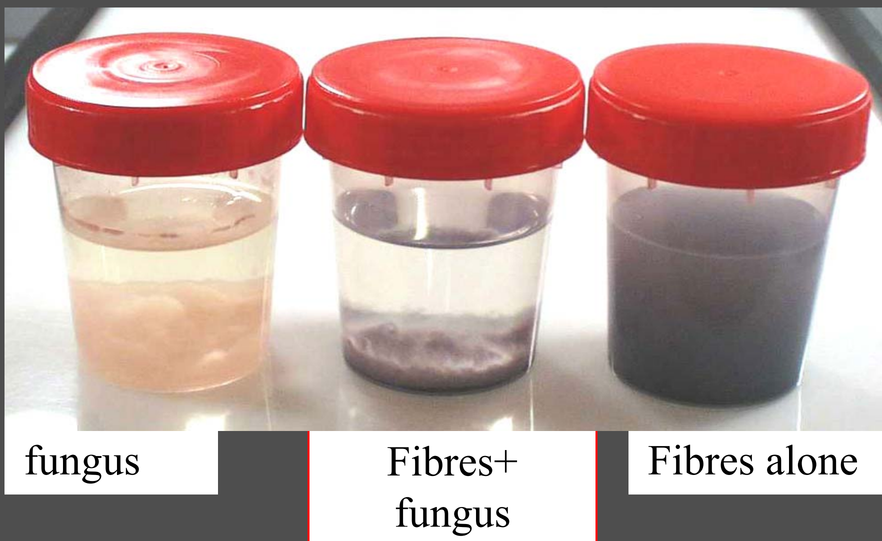
Following iron diffusion, the silica framework collapses

How long may the process proceed?

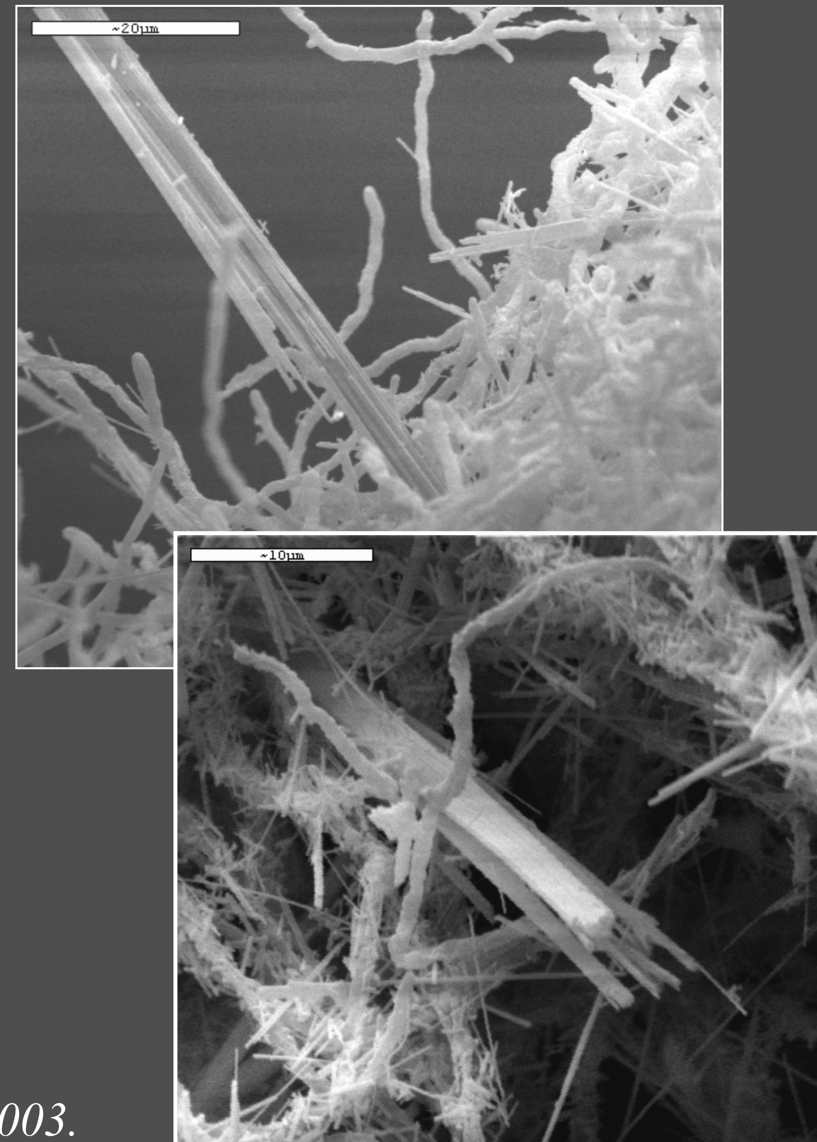
What about microorganisms releasing chelators?

growth of fungi in the presence of asbestos

O-maius E + crocidolite fibres



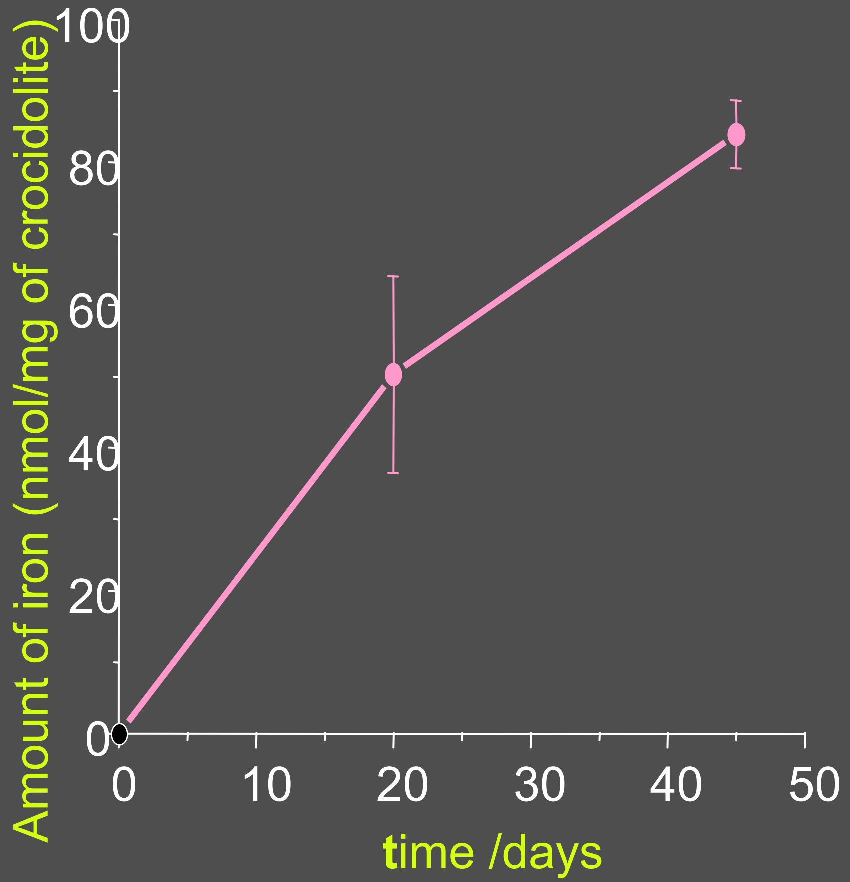
Oidiodendron maius Zn

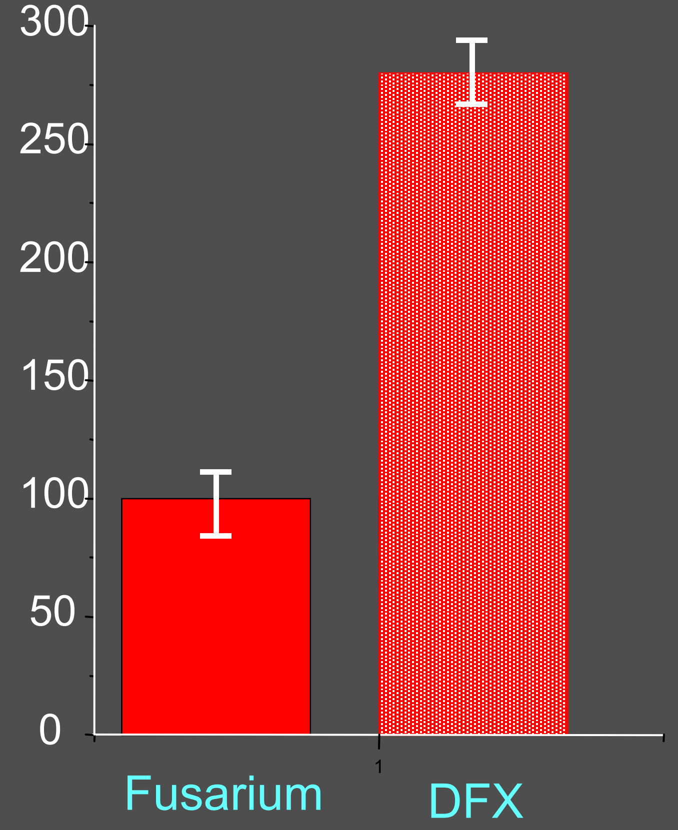


intimate interaction between fungal hyphae and fibers

Martino et al. Angew. Chem. Int. Ed. 42, 219-222, 2003.

iron released in the presence of *Fusarium,* a fungus which produces siderophores in the presence of crocidolite asbestos



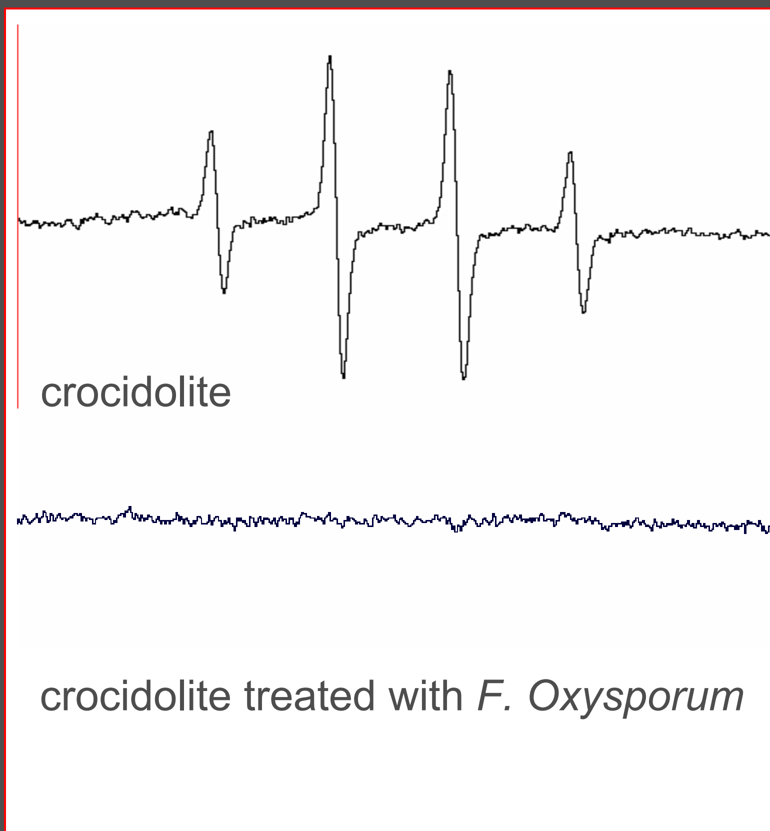


Iron released is comparable to what mobilized by desferrioxamine

Martino et al. Angew. Chem. Int. Ed. 42, 219-222, 2002

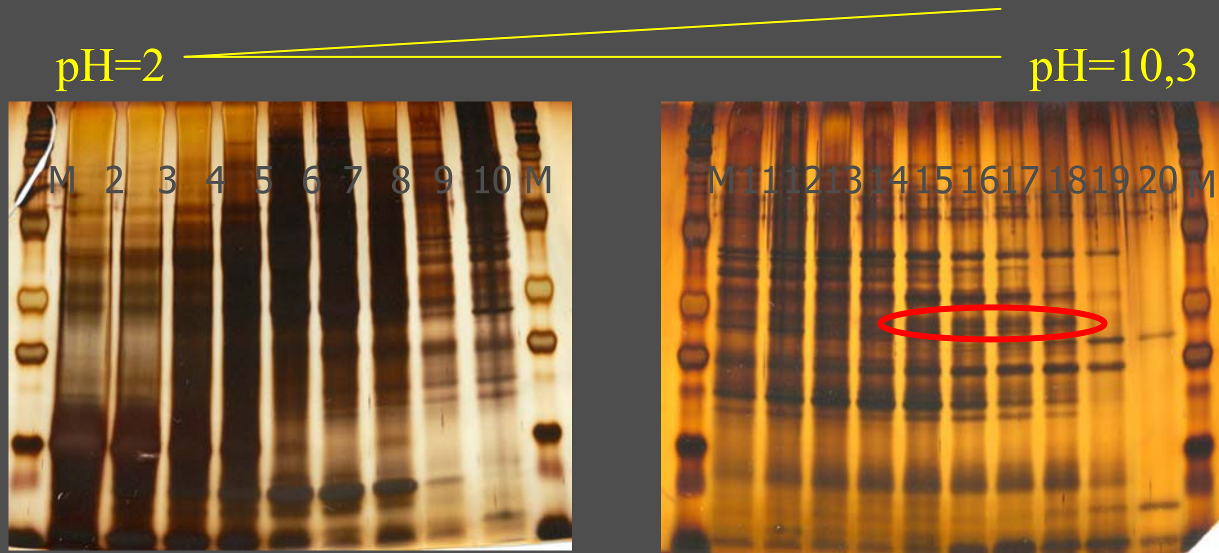
the potential of the fibres to generate free radicals is fully suppressed

Fenton-like reaction
· OH from H_2O_2



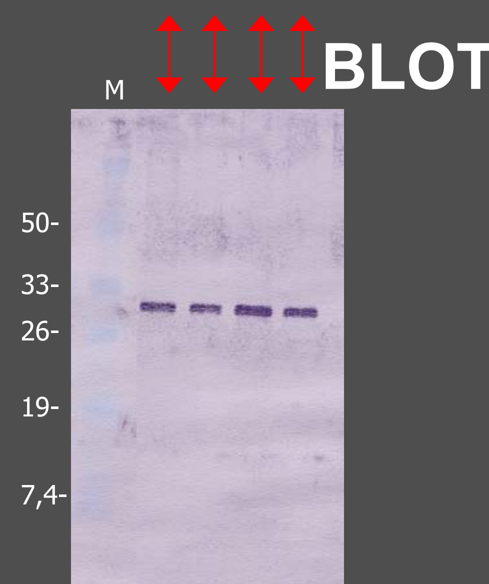
EPR spectra of [DMPO-OH°]

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



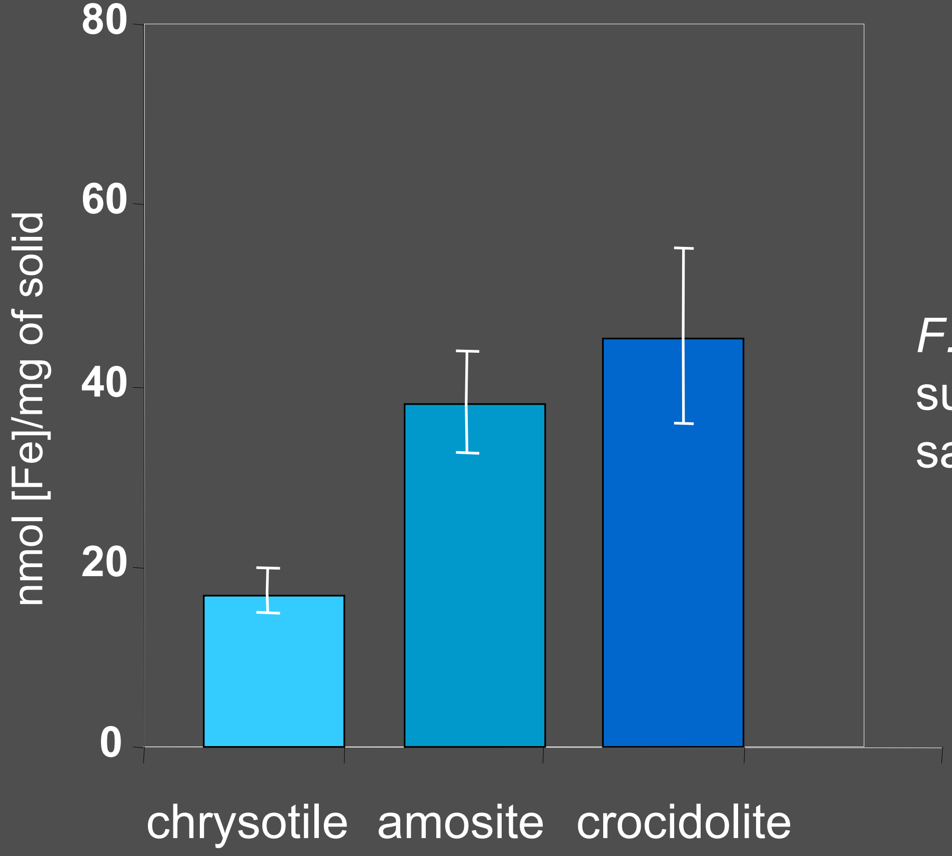
filtered culture medium

extra-cellular proteins



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*

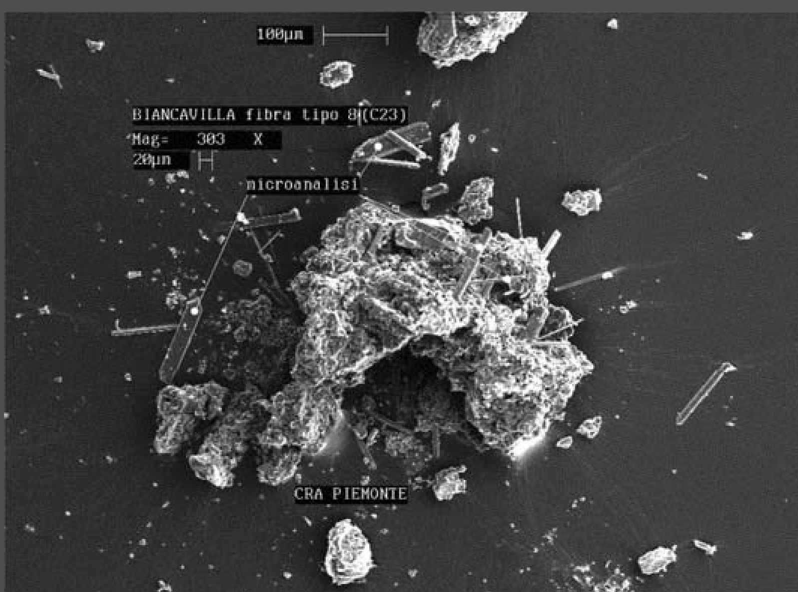
How to predict the toxicity of other asbestiform minerals

→ *from chemistry to pathology and no more from epidemiology up the ladder to mechanisms*

New fibers: the case of Biancavilla (CT,Sicily, Italy)

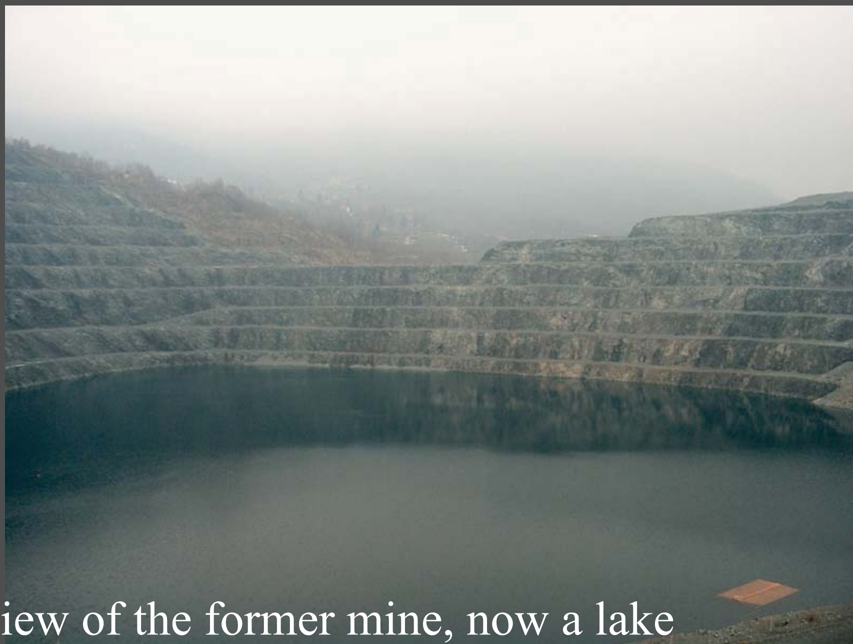
23 cases of pleural mesothelioma, up to 2001, with no professional exposure to asbestos

Tremolite- Actinolite / Fluoroedenite

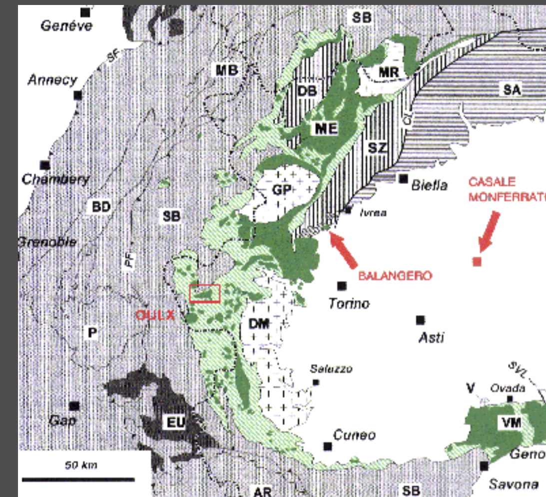


Paoletti et al., Arch.Envir. Health, 55,392, 2000

Balangeroite from the Balangero mine (Piedmont. I)



View of the former mine, now a lake



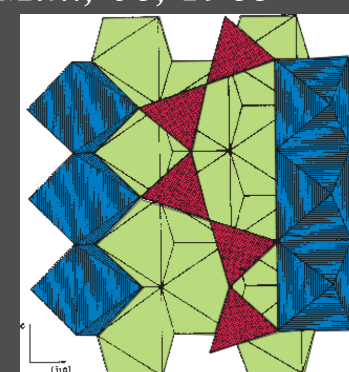
$M_{21}O_3(OH)_{20}(Si_4O_{12})_2$

where M is Mg, Fe(II) and Fe(III)
traces of Mn, Al, Ca, Cr, Ti

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

Natural rock fragment with balangeroite fibers

Long fibers of balangeroite



Balangeroite structure (110 face)

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