Biological Durability and Oxidative Potential of Man-Made Vitreous Fibres as Compared to Crocidolite Asbestos Fibres

Susanne Hippeli[a,*], Kerstin Dornisch[a], Thorsten Wiethege[b], Adrian Gillissen[c], Klaus-Michael Müller[b] and Erich F. Elstner[a]

[a] Lehrstuhl für Phytopathologie, Labor für Biochemische Toxikologie, Technische Universität München-Weihenstephan, D-85350 Freising-Weihenstephan, Germany. Fax: (49) (8161) 714538. E-mail: S.Hippeli@lrz.tum.de

[b] Berufsgenossenschaftliche Kliniken Bergmannsheil, Universitätsklinik, Institut für Pathologie, Bürkle-de-la-Camp-Platz 1, D-44789 Bochum, Germany

[c] Medizinische Universitätsklinik und Poliklinik II, Innere Medizin mit Schwerpunkten Kardiologie, Pneumologie, Sigmund Freud Str. 25, D-53105 Bonn

* Author for correspondence and reprint requests

Z. Naturforsch. **56 c,** 633–648 (2001); receiced January 22/February 12, 2001

Biodurability, Mineral Fibres, Oxidative Potential

In this study we investigated relationships between redox properties and biodurability of crocidolite asbestos fibres and three different man-made vitreous fibres (MMVF): traditional stone wool fibres (MMVF 21), glass fibres (MMVF 11) and refractory ceramic fibres (RCF). Each fibre type was incubated up to 22 weeks in four different incubation media: gamble solution (GS) pH 5.0 and pH 7.4, representing blood plasma without proteins, and surfactant-like solution (SLS) pH 5.0 and pH 7.4. During incubation time aliquots of incubation mixtures were removed and analysed in a biochemical model reaction, mimicking activated phagocytes. In addition, changes of fibre morphology and chemical composition were examined using SEM- and EDX-technology.

In the presence of crocidolite asbestos fibres and MMVF 21 the formation of $OH^{\cdot}$-radicals according to the Haber-Weiss sequence could be demonstrated, whereas MMVF 11 and RCF showed no reactivity. Crocidolite asbestos fibres exhibited a significant higher activity compared with the stone wool fibres at the onset of incubation. The oxidative capacities of these fibre types were shown to depend on both specific surface area and iron content. The oxidative potentials of crocidolite asbestos fibres as well as MMVF 21 were not constant during incubation over several weeks in each incubation medium. The reactivities showed sinoidal curves including reactivities much higher than those at the onset of incubation time. These irregular changes of oxidative capacity may be explained by changes of the redox state of fibre surface-complexed iron.

Furthermore our results showed clear differences between incubation of fibres in GS and SLS, respectively, indicating that phospholipids play an important part in fibre dissolution behaviour and oxidative reactivity.

In conclusion we suggest, that biodurability testing procedures should not exclusively concentrate on dissolution rates of fibres. They should include fibre characteristics concerning known pathogenic mechanisms to evaluate the real toxic potential of the fibre type looking at. Secondly we suggest, that phospholipids should be constituents of incubation liquids used for standardised fibre biodurability test procedures thus representing more realistic incubation conditions.

Introduction

In a previous short communication we reported on redox properties and biodurability of crocidolite asbestos fibres and an experimental stone wool fibre incubated in Gamble solution (GS) and reconstructed surfactant fluid (SLS) (Hippeli *et al.*, 1997).

Redox properties were examined in the NADH/diaphorase/EDTA system. In further investigations we could demonstrate that different types of asbestos fibres can "couple" to enzymatic reactions *in vitro* mimicking the situation found in the proximity of activated phagocytes (Elstner *et al.*, 1986, 1988). One of these enzymatic reactions represents the generation of $O_2^{\cdot -}$, catalyzed by diaphorase in the presence of NADH as electron donor. $O_2^{\cdot -}$ spontaneously dismutates to H_2O_2 and molecular oxygen. $O_2^{\cdot -}$ and H_2O_2 are the main products of the respiratory burst of

0939–5075/2001/0700–0633 $ 06.00

activated phagocytes. From the influence of certain iron chelators (for example EDTA) it has been concluded, that asbestos fibres were able to generate OH·-radicals from $O_2^{\cdot -}$ and H_2O_2 via the catalysis of iron (Haber-Weiss reaction) as an integral part of the asbestos complex (Mossman and Landesman, 1983; Weitzman and Graceffa, 1984; Lund and Aust, 1990; Kamp *et al.*, 1992; Hardy and Aust, 1995). In view of these facts we used the NADH/diaphorase/EDTA system to determine the potential toxicity of asbestos and man made mineral fibres in form of their oxidative potential.

During a time-scale of several weeks these redox properties of the incubated crocidolite asbestos and stone wool fibres undergo irregular changes including reactivities much higher than those at the beginning of the incubation. Two mechanisms have been suggested to be separately or cooperatively responsible for these observed effects: first, changes of the redox states of transition metal ions, where oxidations of reactive ions on the surface are responded by a decrease in redox activity and second, some form of surface "desquamation" during which different types of metal ions could be dissolved or exposed on new surface layers.

In the present study the above mentioned hypotheses of mechanisms have been verified. Four different types of fibres were subjected to comparative investigations: crocidolite asbestos fibres, refractory ceramic fibres (RCF), traditional stone wool fibres (MMVF 21) and the glass fibre MMVF 11. Pathogenicity, biopersistence and biodurability of these fibre types are well documented (Muhle *et al.*, 1998; Kamstrup *et al.*, 1998; Bernstein *et al.*, 1995, 1996; Bellmann *et al.*, 1995; Eastes and Hadley, 1995; Hesterberg *et al.*, 1993, 1998; Alexander *et al.*, 1994; Musselman *et al.*, 1994; Greim 1993; McConnell *et al.*, 1994; Mast *et al.*, 1994; Wilson *et al.*, 1999). Each fibre type was incubated in four different incubation media (GS pH 5.0 and 7.4; surfactant like solution (SLS) pH 5.0 and 7.4) up to 22 weeks. During incubation time aliquots of the incubation mixtures were removed and analysed in the NADH/diaphorase/EDTA system. In addition alterations of fibre structure and chemical composition were examined.

Materials and Methods

Fibre characteristics

Crocidolite asbestos fibres were obtained from stocks originally prepared and characterized by the UICC (Union Internationale Contre le Cancer, Timbrell and Rendall, 1971/72) and purchased from PLANO W. Plannet GmbH, Ernst-Befort-Str. 12, 35578 Wetzlar, Germany.

The basalt-based stone wool fibre (designated MMVF 21) and the glass fibre (designated MMVF 11) were a gift from Prof. Dr. K.-M. Müller (Berufsgenossenschaftliche Kliniken Bergmannsheil – Universitätsklinik, Institut für Pathologie, Bochum, Germany). These fibres are size-separated fine respirable fractions both originally characterized and obtained from the TIMA (Thermal Insulation Manufacturers Association) repository, c/o Mountain Tech. Center, Attn. T. Hesterberg, P. O.Box 5108, Denver CO, 80217–5108, USA.

The refractory ceramic fibre (RCF, product designation ISOBLOCK-Watte 143) was purchased from FMB GmbH Hochtemperaturtechnik, Am Trippelsberg 71, 40589 Düsseldorf, Germany. The original material was milled with a mikro-dismembrator (Type 835162/5 Braun Melsungen, Germany). The milled material was characterized by the Fraunhofer Institute for Ceramic Technologies and Sintered Material (IKTS) Dresden, Winterbergstr. 28, 01277 Dresden, Germany. The specific surface areas of RCF, MMVF 21 and MMVF 11 were also determined by the IKTS according to Brunauer *et al.*, 1938.

The chemical and physical characteristics of the stock fibre materials are given in Table I.

Reagents

KMB (α-keto-γ-methiol-butyric acid), β-NADH, phosphatidylcholine (contaminated with 15% cholesterol; 5% phosphatidylethanolamine, 2% sphingomyelin), superoxide dismutase (SOD), bovine serum albumine (BSA), formate and desferrioxamine were obtained from Sigma, Munich, Germany; Diaphorase was purchased from Boehringer, Mannheim, Germany; EDTA (ethylenediaminetetraacetic acid) and catalase were from Merck, Darmstadt, Germany. All other chemicals were of the highest grade of purity available (Merck). The gases for gas chromatography were

Table I. Chemical and physical fibre characteristics.

	Crocidolite	Refractory Ceramic fibres	MMVF 21 (stone wool)	MMVF 11 (glass fibres)
Chemical composition (in Weight%)				
SiO_2	50.87	46	46.2	63.4
Al_2O_3	0.05	34	13.0	3.88
Fe_2O_3	21.41		7.0	0.25
TiO_2	0.01		2.95	0.06
CaO	0.71		16.9	7.45
MgO	3.41		9.25	2.82
Na_2O	5.62		2.64	15.45
K_2O	0.07		1.25	1.32
MnO	0.05		0.16	0.01
B_2O_3				4.45
ZrO_2		19	0.03	0.03
Diameter range [μm]	0.1–1.3	1–12	0.2 – 6.1	0.08–4.2
AMD ± SD [μm]	0.3 ± 0.2	3.06±2	1.3 ± 0.8	0.9±0.7
Length range [μm]	1.3–30.7	10–400	1.8–76.9	1.7–98.8
AML ± SD [μm]	9.9 ± 7.8	57.6±47.2	24.6 ± 19.9	19 ± 18.7
Spec. surface area [m^2/g]	8.3 ± 0.5	0.166 ± 0.003	1.106 ± 0.009	1.046 ± 0.014

Note: AMD, arithmetic mean diameter; AML, arithmetic mean length; SD, standard deviation.

from Messer, Griesheim, Germany (N_2: type 5.0; H_2: type 5.0; synth. air; ethene calibration gas).

Incubation media and incubation conditions

The fibre materials (80 mg/20 ml) were incubated in two different incubation media: the Gamble solution (GS) (exact composition is described by Scholze and Conradt, 1987), representing the blood plasma without proteins, and the surfactant-like solution (SLS). SLS was prepared by adding a phosphatidylcholine solution (1 g phosphatidylcholine in 2 ml ethanol) to GS (total volume 50 ml). The pH of each of the two incubation media was adjusted to 5.5 and 7.4 with 1 N HCl.

Incubation mixtures were shaken continuously in a water bath at 37 °C. At defined times of incubation (short time experiment: 0, 3, 6, 24, 48, 72, 96, 168 h; long time experiment: during a time scale of 22 weeks (SLS) or 18 weeks (GS), every two weeks) 50 μl were taken from incubation mixtures in order to examine the oxidative potential of the incubated fibre material in the NADH/diaphorase/EDTA system.

NADH/diaphorase/EDTA system and gas chromatographic ethene determination

The enzyme-catalyzed and fibre-stimulated formation of reactive oxygen species was detected as ethene release from α-keto-γ-methiol-butyric acid (KMB). Ethene formation from KMB was analyzed by gas chromatography as described previously (v. Kruedener *et al.*, 1995; Hippeli *et al.*, 1997). The values for ethene production refer to picomol per total reaction and were calculated with the aid of an ethene calibration gas: 1 ml = 235.15 pmol, 1 bar. The reaction mixtures contained in a total volume of 1 ml: 100 mM phosphate buffer (pH 7.4), 75 μM NADH, 1.1 U diaphorase, 1 mM EDTA, 1.5 mM KMB and 50 μl of the incubation mixtures. One unit of diaphorase will oxidize 1.0 μmol of β-NADH per min at pH 5.7 at 25 °C, with the corresponding reduction of the electron acceptor. In order to study reaction mechanisms SOD or catalase (50 U), BSA (12 μg; equivalent to the protein content of 50 U catalase), desferrioxamine (1 mM) or formate (1 mM) were added to the reaction mixtures. One unit of SOD will inhibit the rate of reduction of cytochrome c by 50% in a coupled system with xanthine and xanthine oxidase at pH 7.8 at 25 °C in

a 3 ml reaction volume; one unit of catalase will decompose 1.0 µmole of H_2O_2 per min at pH 7.0 at 25 °C.

At the beginning and at the end of the long term experiment 2 ml of each incubation mixtures were centrifuged for 10 min at 13.000 rpm (Centrifuge 5810, Eppendorf, Hamburg, Germany). Supernatants were quantitatively recovered, fibre pellets were resuspendet in 2 ml of the corresponding incubation medium. Aliquots of 50 µl each were analysed in the NADH/diaphorase/EDTA system.

SEM- and EDX-analysis of stock and incubated fibre materials

Alterations of fibre structure and chemical composition were observed by scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) analyses as described by Respondek *et al.* (1995). Samples of 100 µl incubation mixtures (containing 0.4 mg fibres) were diluted in 5 ml aqua dest., placed on membrane filters (filter type 0.2 µm GTTP, Millipore, Eschborn, Germany) using vacuum and coated with carbon. The coated samples were studied with SEM (DSM 940 Zeiss, Oberkochen, Germany) and EDX (AN 10/25S Link, Oxford, Great Britain) using 10–30 kV.

Results

Two sets of experiments have been performed in order to elucidate the development of potential toxicity of different fibres in solution. The first set of experiments concerns tests within a time scale of 168 hours (short term experiment) whereas the second set concerns a time scale of 18 weeks for incubation in GS and a time scale of 22 weeks in the case of incubation in SLS (long term experiment). As shown in Fig. 1 (short term experiment)

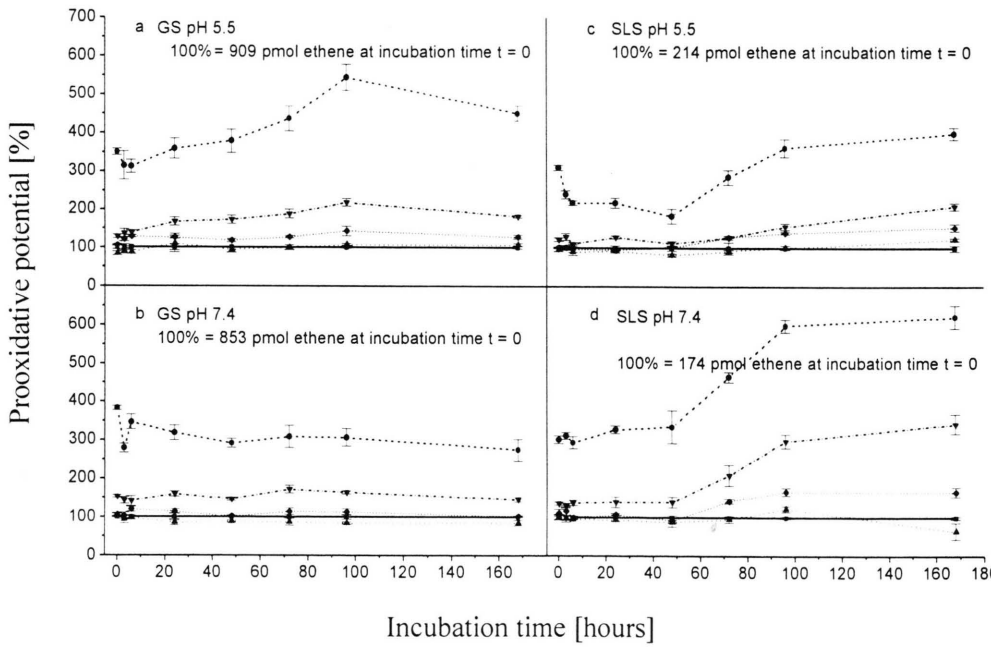

Fig. 1a-d. Short-time experiment: The prooxidative potentials of crocidolite asbestos fibres, MMVF 21, MMVF 11 and RCF in the NADH/diaphorase/EDTA system.
-■- control; -●- crocidolite; -▼- MMVF 21; -◆- MMVF 11; -▲- RCF.
Reaction mixtures contained in a total volume of 1 ml 100 mM phosphate buffer, 75 µM NADH, 1.1 U diaphorase, 0.5 mM EDTA, 1.5 mM KMB and 50 µl incubation mixture. Incubation mixtures consisted of either incubation medium (control) or incubation medium + fibre materials. Incubation media are GS (pH 5.5 and 7.4; Fig. 1a/b) or SLS (pH 5.5 and 7.4; Fig. 1 c/d). Standard deviations represent $n = 6$.
To compare the reactivities of the different fibre types, the ethene formation of the control reaction (incubation medium without fibres) was set as 100%. The ethene formations of the reactions in the presence of fibres were calculated as % of the control reaction.

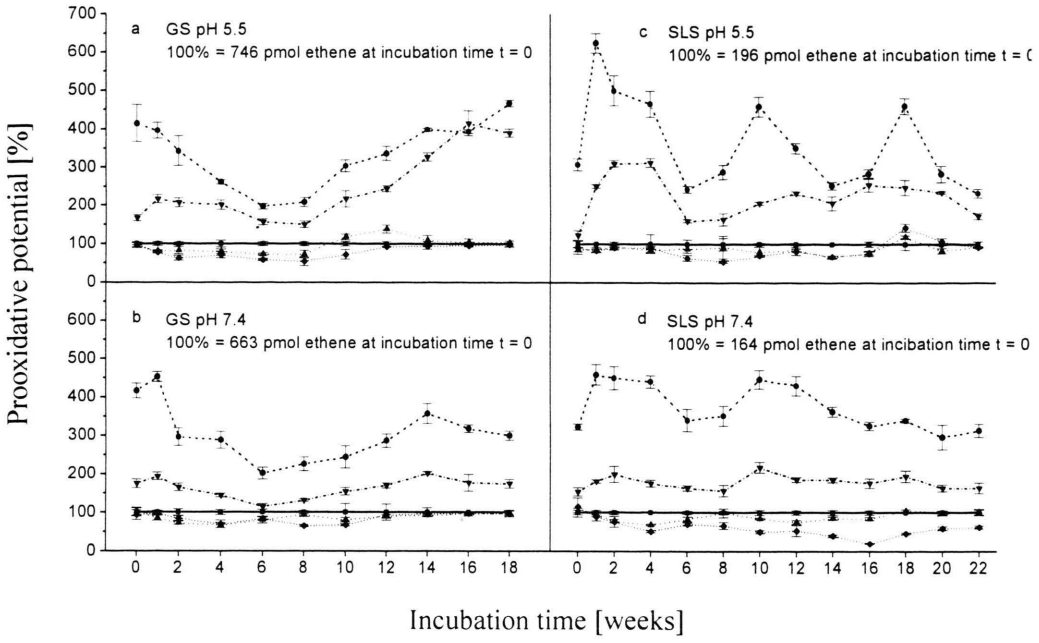

Fig. 2a-d. Long-time experiment: The prooxidative potentials of crocidolite asbestos fibres, MMVF 21, MMVF 11 and RCF in the NADH/diaphorase/EDTA system.
-■- control; -●- crocidolite; -▼- MMVF 21; -◆- MMVF 11; -▲- RCF.
Reaction mixtures and incubation media were as described for Fig. 1a-d.

and Fig. 2 (long term experiment) clear differences between different fibre types can be shown:

In the presence of crocidolite asbestos fibres and stone wool MMVF 21 strong stimulation of ethene release from KMB, triggered by the NADH/diaphorase/EDTA system was observed, whereas RCF and MMVF 11 showed no or slight effects. In order to compare the reactivities of the different fibre types, ethene formation of the control reaction (incubation medium without fibres added to the NADH/diaphorase/EDTA system) was set as 100%. The corresponding pmol values are indicated in the figures. Ethene formation of the reactions in the presence of fibres were calculated as% of the control reaction.

Crocidolite exhibited a significant higher activity as compared to MMVF 21, both in Gamble solution (Fig. 1 a/b and Fig. 2 a/b) and in SLS (Fig. 1 c/d and Fig. 2 c/d). The oxidative potentials of both types of fibres were not constant during incubation up to 22 weeks either in GS or in SLS. Especially in the long term experiment reactivities showed irregular changes including reactivities much higher than those at the onset of incubation. These findings are in agreement with our previous findings (Hippeli *et al.*, 1997).

As demonstrated in Fig. 3a ethene release, triggered by the NADH/diaphorase system, was inhibited by both SOD and catalase, whereas BSA (protein control) showed no effect. EDTA caused an increase in ethene formation. The chelator EDTA strongly enhances certain oxidative processes by facilitating Fe^{3+}-reduction as well as electron transfer from Fe^{2+} to H_2O_2 thus allowing the formation of $OH^{\cdot}$-radicals according to the Haber-Weiss sequence. Increasing ethene release of the NADH/diaphorase system in the presence of EDTA is due to ubiquitary iron impurities.

In Fig. 3b the effects of the investigated fibre types, incubated in GS pH 5.5 for 1 hour, on the NADH/diaphorase system were demonstrated. Crocidolite and to a much lower degree MMVF 21 enhanced the ethene release. In contrast, RCF and MMVF 11 were unreactive. Addition of EDTA to the asbestos-stimulated NADH/diaphorase system resulted in a strong increase of ethene release, as expected (Fig. 3c). Both superoxide dismutase (SOD) and catalase inhibited the reaction

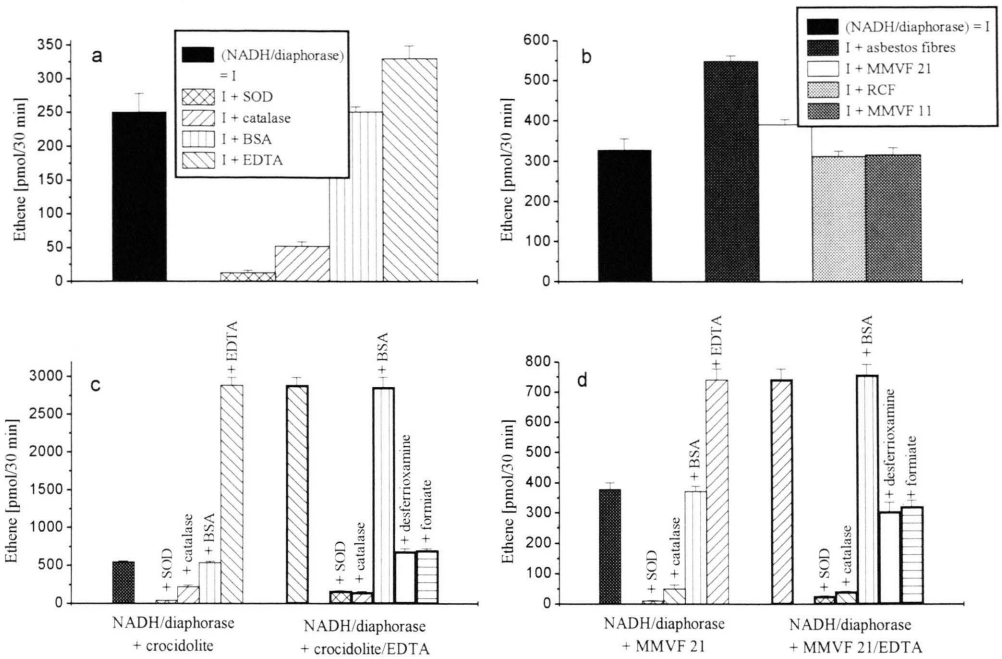

Fig. 3a-d. Ethene release from KMB in the NADH/diaphorase system
Reaction mixtures contained in a total volume of 1 ml 100 mM phosphate buffer, 75 µM NADH, 1.1 U diaphorase, 1 mM KMB, 50 U SOD or catalase, 12 µg BSA, 0.5 mM EDTA or desferrioxamine or formiate, 200 µg crocidolite asbestos fibres or MMVF 21.
Fig. 3a. Ethene release in the NADH/diaphorase system (simulation of the respiratory burst) and the influence of SOD, catalase, BSA and EDTA on the ethene formation.
Fig. 3b. Influence of crocidolite asbestos fibres, MMVF 21, RCF and MMVF 11, incubated in GS pH 5.5 for 1 h, on the ethene release from KMB in the NADH/diaphorase system.
Fig. 3c-d. Influence of SOD, catalase, BSA, EDTA, desferrioxamine and formiate on the ethene release in the presence of crocidolite asbestos fibres (Fig. 3c) and MMVF 21 (Fig. 3d).

of the model system in the presence of asbestos and EDTA. Desferrioxamine, a chelator forming an unreactive complex with Fe^{3+}-ions, suppressed the crocidolite stimulated ethene release of the enzyme system. These results clearly indicate an iron-mediated formation of reactive oxygen species via the Haber-Weiss reaction type. As shown in Fig. 3d MMVF 21 exhibited comparable reaction patterns, underlining the role of iron in this fibre toxicity. The unreactivity of RCF and MMVF 11 in these model reaction could be explained by lacking iron (RCF) or by a too low iron content (MMVF 11) (see Table I).

A fibre-dose response is given in Fig. 4. Crocidolite asbestos fibres (Fig. 4a) and MMVF 21 (Fig. 4b) were incubated at different concentrations in each of the incubation media for 1 hour and than tested in the NADH/diaphorase/EDTA system.

The reactivities were generally lower in SLS. The only distinction between GS and SLS is the content of phospholipids in SLS, which may in part act as radical scavengers for reactive oxygen species formed by the NADH/diaphorase/EDTA-system. The oxidative potentials of crocidolite and less pronounced of MMVF 21 were higher at pH 5.5 compared to pH 7.4, especially in GS.

Crocidolite asbestos fibres contain iron three times higher than those of MMVF 21. Concerning iron-content, the reactivity of 200 µg stone wool fibres has to be compared with the reactivity of 67 µg asbestos fibres. The specific surface area of 200 µg MMVF 21 comprehends 2.212 cm^2, that of 67 µg asbestos fibres 5.478 cm^2 (data calculated from Table I). Calculated reactivities corresponding to a specific surface area of 1 cm^2 were summarized in Table II. These data clearly indicate, that

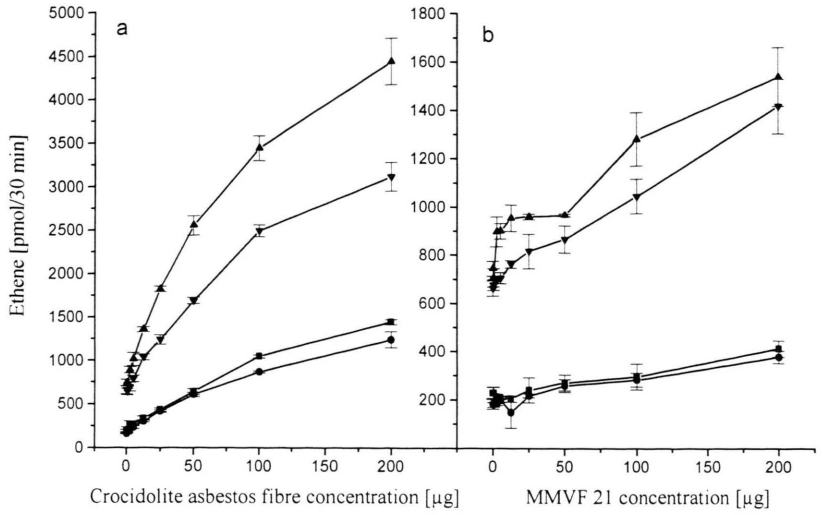

Fig. 4a-b. Ethene release in the NADH/diaphorase/EDTA system in the presence of different fibre concentrations of crocidolite asbestos (Fig. 4a) and MMVF 21 (Fig. 4b), incubated in -▲-GS pH 5.5; -▼-GS pH 7.4; -■-SLS pH 5.5 and -●-SLS pH 7.4 for 1 h.

Table II. Reactivities of crocidolite asbestos fibres and man-made vitreous fibres 21 in the NADH/diaphorase/EDTA system, adjusted to iron content and surface area.

	Crocidolite asbestos fibres 67 µg / 5.478 cm^2 spec. surface area		MMVF 21 200 µg / 2.212 cm^2 spec. surface area	
	pmol ethene	pmol ethene adjusted to 1 cm^2 spec. surface area	pmol ethene	pmol ethene adjusted to 1 cm^2 spec. surface area
GS pH 5.5	2890	528	1212	548
GS pH 7.4	2000	365	1418	641
SLS pH 5.5	820	150	377	170
SLS pH 7.4	690	126	305	137

the enhancement of ethene release from KMB in the NADH/diaphorase/EDTA system in the presence of crocidolite or MMVF 21 is a function of surface area and iron content. Adjustment to both equal iron content and equal surface area yielded comparable ethene formation at the beginning of incubation in each incubation medium, with the exception of GS pH 7.4.

Data in Table III showed that at the onset of incubation (1 hour) the observed reactivities of crocidolite as well as MMVF 21 are exclusively due to the presence of fibres. Samples of the incubation mixtures were removed after one hour incubation time and centrifuged in order to separate incubation media plus possibly leached elements from fibre material. Fibre pellets were resuspended in corresponding incubation media. Aliquots of each were tested in the NADH/diaphorase/EDTA system and compared with total reactivity. All supernatants showed reactivities comparable to control reaction (= 100%). Oxidative potentials of resuspended fibres corresponded to total reactivities. Results of the same experiment carried out at the end of long term incubation were also shown in Table III. In the case of crocidolite reactivities retained associated with fibre material. In contrast, "MMVF 21-supernatants" of near neutral incubation media GS and SLS exhibited significant enhanced reactivities, whereas the corresponding resuspended fibre pellets showed reduced activities compared with total reactivity, indicating a leaching of iron from

Table III. Localisation of reactivity.

Stimulation of the ethene release from KMB in the NADH/diaphorase/EDTA system [% of the control reaction; control reaction = 100%]:	Crocidolite asbestos fibres							
	GS pH 5.5 Time of incubation		GS pH 7.4 Time of incubation		SLS pH 5.5 Time of incubation		SLS pH 7.4 Time of incubation	
	1h	18 weeks	1h	18 weeks	1h	22 weeks	1h	22 weeks
Total reactivity	350 ± 8	480 ± 25	416 ± 19	300 ± 12	308 ± 16	233 ± 12	323 ± 8	314 ± 17
Reactivity of the supernatant	107 ± 4	159 ± 11	103 ± 11	112 ± 6	111 ± 9	106 ± 5	98 ± 2	123 ±8
Reactivity of the resuspended pellet	328 ± 8	408 ± 24	422 ± 24	288 ± 16	289 ± 10	245 ± 22	306 ± 16	331 ± 11
	MMVF 21							
	GS pH 5.5 Time of incubation		GS pH 7.4 Time of incubation		SLS pH 5.5 Time of incubation		SLS pH 7.4 Time of incubation	
	1h	18 weeks	1h	18 weeks	1h	22 weeks	1h	22 weeks
Total reactivity	150 ± 5	390 ± 26	175 ± 12	176 ± 14	122 ± 12	173 ± 8	154 ± 11	164 ± 14
Reactivity of the supernatant	98 ± 10	106 ± 14	98 ± 6	152 ± 7	106 ± 7	109 ± 7	96 ± 4	150 ± 7
Reactivity of the resuspended pellet	149 ± 16	368 ± 11	167 ± 9	131 ± 9	125 ± 2	159 ± 11	144 ± 9	120 ± 3

MMVF 21, but not from asbestos fibres. These assumptions were confirmed by EDX-analyses. The element maps of asbestos fibres (Table IV) showed no significant differences during time scale concerning the ratio of Si to Fe, irrespective of type of incubation medium or pH. In addition SEM-analyses of stock material compared to incubated fibres showed no morphological changes of fibre structure (data not shown).

In the case of MMVF 21 a strong increase of Si to Fe-ratio was observed in GS pH 7.4 (from 8.3 to 18.2) and in SLS pH 7.4 (from 8.3 to 32.6) at the end of long term incubation (Table V). Incubation in the acidic media did not cause any alteration of the Si to Fe-ratio. But similar to crocidolite asbestos fibres no changes in fibre morphology could be demonstrated by SEM during long term incubation in any incubation medium (data not shown).

Looking at the element maps of RCF (Table VI) it was noticeable, that the Zr-content varied to a large extent. Interestingly only in SLS Zr-contents of more than 20% were observed. In addition only in these incubation medium, independent on pH, a destruction of the nucleopore filter membrane accompanied by fibre breaking causing sharp edges was detected by SEM (Fig. 5a-c).

EDX-analyses of the glass fibre MMVF 11 indicated a leaching of Ca, but only by incubation in SLS (Table VII). Incubation in GS yielded no significant changes of the chemical composition of the fibre looking at, although chrystalline formations on fibre surface were observed by SEM (Fig. 6a). Chrystalline formations were also observed on fibre surface of glass fibres incubated in SLS, but to a less amount compared with GS (Fig. 6b).

Discussion

Of utmost importance for fibre toxicity is the geometry of these particles i.e. the ratio between length and diameter. This ratio determines the topographical localisation in the respiratory tract. Another probably equal important property is the chemical composition and, dependent or indepen-

Table IV. Results of the EDX-analyses.

Incubation medium	Elements	Time of incubation (days)					
		0	7	28	56	126	154
		Percentage of elements contained in crocidolite asbestos fibres					
SLS, pH 5.5	Si	53.9	54.1	51.3	46.6		53.4
	Mg	6.1	4.5	5.2	5.2		7.2
	Fe	23.2	25.5	26.7	24.7		25.2
	Na	16.8	15.9	16.7	23.5		22.5
	Mg + Na (A)	22.9	20.4	21.9	28.7		29.7
	Si : A	2.4	2.7	2.3	1.6		1.8
	Si : Fe	2.3	2.1	1.9	1.9		2.1
SLS, pH 7.4	Si	53.9	51.8	51.8	52.1		59.0
	Mg	6.1	3.8	5.2	4.1		6.1
	Fe	23.2	28.9	27.1	27.0		26.3
	Na	16.8	15.5	15.4	15.9		19.6
	Mg + Na (A)	22.9	19.3	20.6	20.0		24.7
	Si : A	2.4	2.7	2.5	2.6		2.4
	Si : Fe	2.3	1.8	1.9	1.9		2.2
GS, pH 5.5	Si	53.9		51.5	52.5	59.3	
	Mg	6.1		4.5	4.5	6.6	
	Fe	23.2		28.3	25.8	24.8	
	Na	16.8		15.7	17.2	19.3	
	Mg + Na (A)	22.9		20.2	21.7	25.9	
	Si : A	2.4		2.5	2.4	2.3	
	Si : Fe	2.3		1.8	2.0	2.4	
GS, pH 7.4	Si	53.9	53.9	50.5	53.4	59.3	
	Mg	6.1	6.1	2.8	3.4	6.0	
	Fe	23.2	23.2	35.4	29.9	26.4	
	Na	16.8	16.8	11.4	13.2	18.3	
	Mg + Na (A)	22.9	22.9	14.1	16.7	24.3	
	Si : A	2.4	2.4	3.6	3.2	2.4	
	Si : Fe	2.3	2.3	1.4	1.8	2.2	

dent of this composition, the biodurability. Biological longevity (biodurability) seems to be tightly correlated with a strong tumor-inducing potential (Stanton and Wrench, 1972). Biodurability was included in addition to geometry as evaluating criterium with regard to this carcinogenic potential. Several uncertainties concerning these criteria are known: ceramic fibres are clearly less soluble than mineral fibres and are only extrem slowly removed from rat lungs (Hammad *et al.*, 1988). In agreement with this fact a high toxicity in animal experiments has been demonstrated (Hesterberg *et al.*, 1992). In comparison to amphibolic asbestos fibres such as the toxic crocidolite, ceramic fibres appear to be less dangerous, however. This may be due to the fact that ceramic fibres, in contrast to amphibolic asbestos fibres, do not split longitudinally. The overall toxicity is apparently not only determined by geometry and durability: chemical reactivity of atoms (ions) or molecule-complexes exposed to the surface are additional parameters in this respect (Fubini 1993; Martra *et al.*, 1999).

Most investigations concerning biodurability have been conducted *in vivo* where the number of countable fibres present in the lung after a certain time have been used as main parameter for durability. This value has been correlated with toxicity. The number of fibres present for longer time in the lung is dependent on their length. Short fibres undergo rapid phagocytosis and upward transport by alveolar macrophages (Morgan and Holmes, 1984). Long fibres may be broken down yielding shorter ones which than are also transported by macrophages (Bernstein *et al.*, 1995). Only in the

Table V. Results of the EDX-analyses.

Incubation medium	Elements	Time of incubation (days)						
		0	7	28	56	98	126	154
		Percentage of elements contained in MMVF 21						
SLS, pH 5.5	Si	43.7	43.2	44.8	40.0			42.1
	Al	16.5	15.2	15.9	14.8			15.6
	Mg	14.1	13.1	13.0	12.9			12.4
	Fe	5.2	4.3	4.5	5.0			5.0
	Ca	18.0	16.2	18.1	16.4			16.3
	Na	5.0	4.8	n.d.	6.3			5.0
	K	1.3	1.4	1.0	2.5			1.6
	Ti	1.9	1.8	2.7	2.1			2.0
	Mg + Na + K + Ca (A)	38.4	35.4	32.1	38.1			35.3
	Si : A	1.1	1.2	1.4	1.0			1.2
	Si : Fe	8.3	10.0	10.0	8.0			8.4
SLS, pH 7.4	Si	43.7	41.9	41.6	41.9	44.4		52.9
	Al	16.5	15.1	15.5	14.9	13.9		17.3
	Mg	14.1	14.2	13.0	13.1	11.5		14.5
	Fe	5.2	4.7	5.1	5.2	5.6		1.6
	Ca	18.0	14.0	16.2	16.3	15.8		11.5
	Na	5.0	6.3	5.8	5.5	6.2		
	K	1.3	1.2	1.1	1.3	1.8		1.2
	Ti	1.9	1.8	1.7	1.8	2.7		1.0
	Mg + Na + K + Ca (A)	38.4	35.8	36.1	36.2	33.4		27.2
	Si : A	1.1	1.2	1.2	1.2	1.3		1.9
	Si : Fe	8.3	8.8	8.2	8.1	7.9		32.6
GS, pH 5.5	Si	43.7	42.6	39.9	43.0		42.1	
	Al	16.5	15.2	15.2	15.2		13.8	
	Mg	14.1	13.5	13.0	13.6		12.8	
	Fe	5.2	4.4	5.3	4.5		5.2	
	Ca	18.0	15.5	17.3	14.7		17.6	
	Na	5.0	5.2	5.8	5.6		4.7	
	K	1.3	1.3	1.5	1.4		1.6	
	Ti	1.9	1.8	2.1	1.9		2.1	
	Mg + Na + K + Ca (A)	38.4	35.6	37.5	35.3		36.7	
	Si : A	1.1	1.2	1.1	1.2		1.2	
	Si : Fe	8.3	9.6	7.5	9.5		8.0	
GS, pH 7.4	Si	43.7	41.8	41.6	42.6		41.8	
	Al	16.5	15.5	15.4	15.3		15.9	
	Mg	14.1	13.9	11.9	13.6		13.0	
	Fe	5.2	4.6	5.5	4.3		2.3	
	Ca	18.0	14.5	16.0	15.9		15.3	
	Na	5.0	6.7	5.6	5.3		4.8	
	K	1.3	1.2	1.6	1.0		1.6	
	Ti	1.9	1.8	2.4	1.9		2.2	
	Mg + Na + K + Ca (A)	38.4	36.3	35.1	35.9		34.7	
	Si : A	1.1	1.5	1.2	1.2		1.2	
	Si : Fe	8.3	9.1	7.5	9.8		18.2	

case where fibres are to long to be phagocytosable the retention time in the lung depends on their solubility. *In vivo* and *in vitro* investigations show identical durability of those fibres (Eastes *et al.*, 1995). In summary the situation seems to be even more complicated: the biodurability of fibres in the lung depends on yet more factors than solely their solubility.

Most *in vitro* investigations concerned mass losses within a certain time as a measure of their

Table VI. Results of the EDX-analyses.

Incubation medium	Elements	Time of incubation (days)						
		0	7	28	56	84	98	154
		Percentage of elements contained in refractory ceramic fibres						
SLS, pH 5.5	Si	59.2	56.3	45.1	56.3	55.6	55.3	49.2
	Al	35.0	33.0	28.3	32.8	34.1	31.8	27.8
	Zr	5.8	10.7	26.6	10.6	10.4	12.8	23.0
SLS, pH 7.4	Si	59.2	56.5	57.1	50.4	56.7		57.1
	Al	35.0	33.1	32.5	27.7	34.5		29.3
	Zr	5.8	10.4	10.4	21.9	8.8		13.6
GS, pH 5.5	Si	59.2	56.3	56.7	57.3	57.5	52.7	
	Al	35.0	32.8	33.1	33.5	35.3	36.1	
	Zr	5.8	10.8	10.3	9.2	7.1	11.2	
GS, pH 7.4	Si	59.2	56.7	58.0	59.2	57.5		
	Al	35.0	33.2	32.8	31.0	30.1		
	Zr	5.8	10.0	9.3	9.7	12.3		

durability (for review see Meringo *et al.*, 1994). *In vivo* investigations on the other hand allow to draw conclusions between potential toxicity and persistence in the respiratory tract. They do not allow, however, correlation between persistence and solubility of the individual fibres. Available data on *in vitro* systems allow conclusions on their solubility but not on the correlation between solubility and toxicity.

The goal of our investigations therefore was to establish biochemical model systems for the continuous measurement of potential toxicity during the process of biodegradation. The main question to be asked was whether a continuous loss of mass is tightly connected with a continuous loss of toxicity. Only in the case of a positive answer to this question it seems feasible to deduce that high solubility is also correlated with low toxicity and vice versa.

The process of biodegradation was simulated by incubation of the fibre materials in artificial media mimicking the lining fluid (GS) and the surfactant (SLS) of the lung.

Using a simple biochemical model reaction, simulating activated phagocytes, the iron mediated formation of strong oxidants in the presence of crocidolite asbestos fibres and MMVF 21 could be demonstrated. The oxidative capacities of these fibre types were shown to depent on both specific surface area and iron content. Crocidolite asbestos fibres possess a three times higher iron-content and an eight times higher specific surface area as compared to MMVF 21. These differences were exclusively responsible for the much stronger oxidative potential of crocidolite asbestos as compared to MMVF 21 on the basis of same fibre mass concentrations at the onset of incubation. The contribution of ROS to overall asbestos toxicity and the role of iron as an integral part of the asbestos complex has been well documented by several groups in the past decade (for review see Hardy and Aust, 1995; Kamp and Weitzman, 1997). Ghio and co-workers, (1992) showed, that the *in vitro* production of hydroxyl radicals by crocidolite asbestos fibres increases with the [Fe^{3+}] complexed to the dust surface. In a later report (Ghio *et al.*, 1994a) the authors pointed out, that structural metal is unlikely to participate in free radical production, but it can catalyse heterogeneous electron transport. Structural oxidation of iron silicates occurs through an electron hopping mechanism. This can produce an increase of ferric cation in the lattice by reduction of surface Fe^{3+}. The surface iron can than be reoxidized by atmospheric oxygen (Fubini and Mollo, 1995). This can faciliate inner sphere electron transfer between structural and adsorbed iron states. These mechanisms of electron transfer ultimately depend on the concentra-

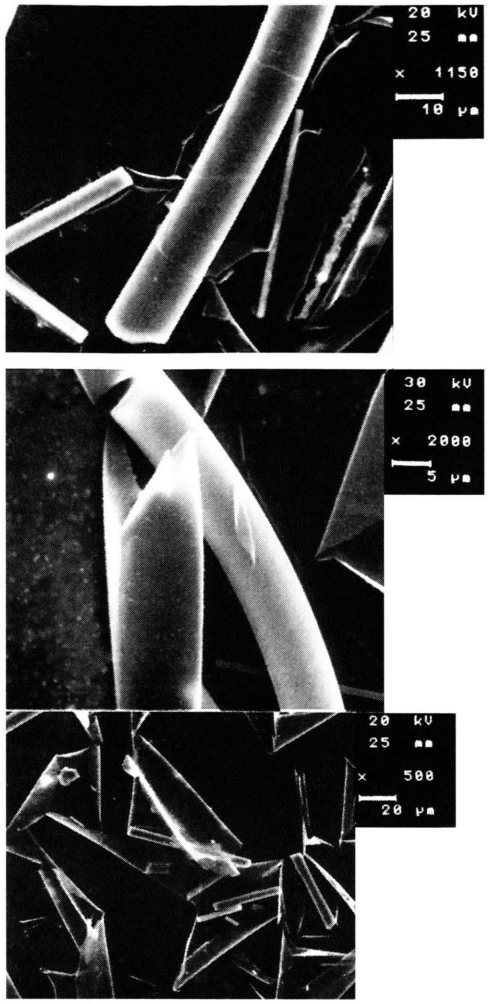

Fig. 5a-c. Breaking and peeling of RCF after incubation in SLS pH 7.4 (a) or SLS pH 5.5 (b) for 22 weeks. Destruction of the membraneous filter by RCF incubated in SLS pH 7.4 for 22 weeks (c).

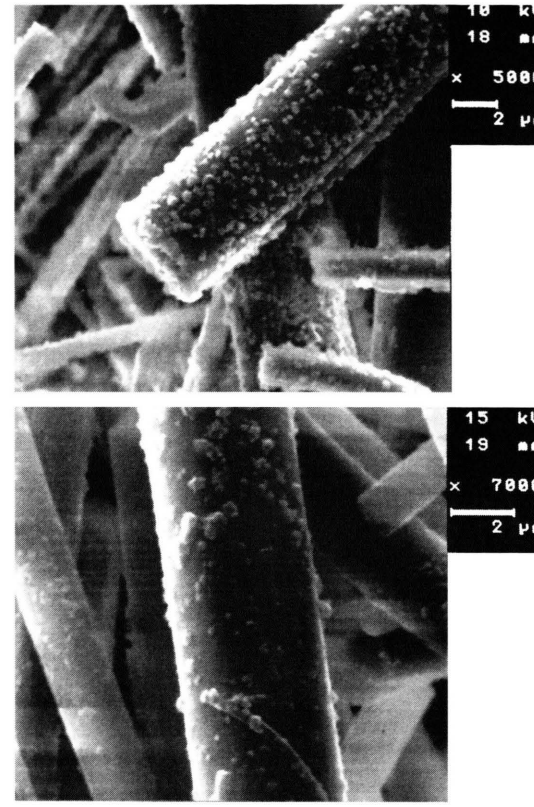

Fig. 6a-b: Coating of MMVF 11 after incubation in GS (a) or SLS (b) for 16 weeks.

tion of surface-complexed iron. The high percentage of SiO_2 in crocidolite asbestos and MMVF 21 and the consequent high density of surface silanol groups in these fibres result in a high capacity to coordinate metal ions on the surface.

Astolfi and co-workers (1991) observed strong changes in the "state and population of iron ions" in different asbestos fibres if they are exposed to a biologically relevant matrix for longer times. Zalma *et al.* (1989) documented that fibres with a completely oxidized surface lose their toxicity. But surface-$[Fe^{3+}]$ can be reduced by $O_2^{\cdot -}$, produced by activated phagocytes (Ghio *et al.*, 1994b). In view of this facts the observed irregular changes of oxidative capacity could be explained with changes of the redox states of transition metal ions, where oxidations of reactive ions on the surface are responded by a decrease in redox activity and reduction of surface ferric iron by an increase of oxidative power.

Ghio and co-workers (1994b) reported, that reduction of ferric ion by superoxide yields in a displacement from the crocidolite asbestos fibre surface. Lund and Aust (1990) showed, that at acidic pH the Fe(II)-mobilisation from crocidolite in the presence of an iron chelator was strongly enhanced as compared to neutral or alkaline pH. These findings could explain the higher oxidative potential at pH 5.5 observed in our incubation experiments, indicating enhanced oxidative capacities of crocidolite and MMVF 21 in the presence of phagocytes due to the lower pH in the microenvironment of activated macrophages and neutrophils. On the other hand damaging effects of oxi-

Table VII. Results of the EDX-analyses.

Incubation medium	Elements	Time of incubation (days)						
		0	7	28	56	98	112	154
		Percentage of elements contained in MMVF 11						
SLS, pH 5.5	Si	61.6	62.9	63.9				64.1
	Al	5.4	5.3	5.1				5.1
	Mg	4.2	4.0	4.0				4.3
	Ca	7.8	7.9	8.5				5.9
	Na	19.4	18.3	16.6				19.1
	K	1.3	1.6	1.8				1.4
	Mg + Na + K + Ca (A)	32.6	31.8	30.9				30.7
	Si : A	1.9	2.0	2.1				2.1
SLS, pH 7.4	Si	61.6	68.5	64.7				64.8
	Al	5.4	6.2	4.0				6.0
	Mg	4.2	3.2	3.8				4.5
	Ca	7.8	9.2	8.1				4.7
	Na	19.4	11.0	17.6				19.0
	K	1.3	1.9	1.8				1.1
	Mg + Na + K + Ca (A)	32.6	25.3	31.3				29.2
	Si : A	1.9	2.7	2.1				2.2
GS, pH 5.5	Si	61.6	62.5	61.0	60.2		60.6	
	Al	5.4	5.1	4.5	4.6		4.4	
	Mg	4.2	4.6	4.0	4.8		6.9	
	Ca	7.8	8.1	12.7	10.5		9.8	
	Na	19.4	18.4	15.1	17.4		16.7	
	K	1.3	1.4	2.5			1.2	
	Mg + Na + K + Ca (A)	32.6	32.4	34.5	32.7		34.5	
	Si : A	1.9	1.9	1.8	1.8		1.8	
GS, pH 7.4	Si	61.6	63.8	63.0	67.6	65.3		
	Al	5.4	5.0	4.6	4.4	3.9		
	Mg	4.2	4.1	4.4	3.7	3.6		
	Ca	7.8	7.8	9.5	10.7	10.8		
	Na	19.4	17.8	16.4	11.7	14.3		
	K	1.3	1.5	2.0	1.8	2.1		
	Mg + Na + K + Ca (A)	32.6	31.2	32.4	27.8	31.1		
	Si : A	1.9	2.0	1.9	2.4	2.1		

dants in the alveolar region can be weakened by oxidation of phospholipids of the surfactant layer (Ghio and Hatch, 1993). As shown in our incubation experiments reactivities of crocidolite and MMVF 21 were generally lower in SLS compared to GS.

For crocidolite, which in our experiments exhibits a high oxidative potential, it was shown by *in vivo* short time experiments (Roggli *et al.*, 1987; Musselman *et al.*, 1994) and by *in vivo* long time experiments (Bellman *et al.*, 1987; Bernstein *et al.*, 1995; Dufresne *et al.*, 1999) that the median diameter of the fibres did not change in the course of the experiment. Our SEM- and EDX-analyses of crocidolite asbestos fibres during incubation showed no morphological and chemical changes. If, however, rock wool fibres are tested in long time experiments the median diameter becomes clearly reduced (Musselman *et al.*, 1994; Dufresne *et al.*, 1999). Our SEM-analysis of incubated MMVF 21 demonstrated no visible changes of the fibre surface, but we did not investigate median diameter. EDX-analysis showed a strong enhancement of the Si : Fe-ratio of fibres incubated in GS or SLS at pH 7.4 at the end of long term experiment, indicating a leaching of iron. This was confirmed by the results of the "supernatant-pellet" experiment, showing significant enhanced reactivi-

ties of supernatants of near neutral incubation media, whereas the corresponding resuspended fibre pellets showed reduced activities compared with total reactivity. In other investigations it was shown that MMVF 21 stone wool is highly insoluble under acidic conditions but slightly soluble in alkaline solutions (Muhle *et al.*, 1998).

RCF and the glass fibre MMVF 11 exhibited no oxidative properties in our model system due to lacking of iron (RCF) or due to a too low iron content (MMVF 11). The used RCF was quite similar to the TIMA-RCF2 (Alexander *et al.*, 1994). In contrast to TIMA-RCF1 (Brown *et al.*, 1998), a kaolin-based refractory ceramic fibre, no data are available about free radical activity of RCF2. Inhalation studies showed, that RCF2 induces pulmonary fibrosis, mesothelioma as well as significant increases in lung tumors (Glass *et al.*, 1992). RCF2 were shown to induce a strong cytotoxical response (Lindgren *et al.*, 1996). This may be due to cellular damage caused by sharp edges of fibres as shown in Fig. 5. Interestingly, fibre splitting accompanied by the formation of sharp edges was only observed when fibres were incubated in SLS. The mechanism of fibre splitting remains to be determined, but this observation emphasises the role of phospholipids in modulating fibre toxicity.

Acknowledgements

This work was financially supported by a grant from the "Land Baden-Württemberg" managed by the project management at the Forschungszentrum Karlsruhe-PUG.

We also thank Dr. I. Schmitz. Dr. F. Brasch and Ms. C. Troske for technical assistance and the performance of EDX- and SEM-analyses.

Astolfi A., Belluso E., Ferraris G., Fubini B., Giamello E. and Volante M. (1991), Asbestiform minerals associated with chrysotile from western Alps (Piedmont, Italy): Chemical characteristics and possible related toxicity. In: Mechanisms in Fibre Carcinogenesis (Brown R. C., Hoskins J. A. and Johnson N. F. eds.). New York, Plenum Press, pp. 269–283.

Alexander I. C., Brown R. C., Jubb G. A., Pickering P., and Hoskins J. A. (1994), Durability of ceramic and novel man-made mineral fibres. Environ. Health Perspect. **102**(5), 67–71.

Bellmann B., Muhle H., Pott F., Konig H., Kloppel H. and Spurny K. (1987), Persistence of man-made mineral fibers (MMMF) and asbestos in rat lungs. Ann. Occup. Hyg. **31**, 693–709.

Bellmann B., Muhle H., Kamstrup O. and Draeger U. F. (1995), Investigation on the biodurability of chemical different stone wool fibres. Exp. Toxic. Pathol. **47**, 195–201.

Bernstein D. M., Morscheidt C., Tiesler H., Grimm H.-G., Thévenaz P. and Teichert U. (1995), Evaluation of the biopersistence of commercial and experimental fibres following inhalation. Inhal. Toxicol. **7**, 1031–1058.

Bernstein D. M., Morscheidt C., Grimm H. G. and Teichert U. (1996), The evaluation of soluble fibers using the inhalation biopersistence model, a nine fiber comparison. Inhal. Toxicol. **8**, 345–385.

Brown D. M., Fisher C. and Donaldson K. (1998), Free radical activity of synthetic vitreous fibers: iron chelation inhibits hydroxyl radical generation by refractory ceramic fiber. J. Toxicol. Environ. Health **53**, 545–561.

Brunauer S., Emmett P. H. and Teller E. (1938), Adsorption of gases in multimolecular layers. J. Amer. Chem. Soc. **60**, 309–319.

Dufresne A., Perrault G., Yamato H., Masse S., Begin R. (1999), Clearance of man made mineral fibres from the lungs of sheep. Occup. Environ. Med. **56**(10), 684–690.

Eastes W. and Hadley J. G. (1995), Dissolution of fibres inhaled by rats. Inhal. Toxicol. **7**, 179–196.

Eastes W., Morris K. J., Morgan A., Launder A. K., Collier C. G. *et al.* (1995), Dissolution of glass fibers in the rat lung following intratracheal instillation. Inhal. Toxicol. **7**, 197–213.

Elstner E. F., Schütz W. and Vogl G. (1986), Enhancement of enzymic catalyzed production of reactive oxygen species by suspensions of crocidolite asbestos fibres. Free Rad. Res. Comun. **1** (6), 355–359.

Elstner E. F., Schütz W. and Vogl G. (1988), Cooperative stimulation by sulfite and crocidolite asbestos fibres of enzyme catalyzed production of reactive oxygen species. Arch. Toxicol. **62**, 424–427.

Fubini B. (1993), The possible role of surface chemistry in the toxicity of inhaled fibres. In: Fibre Toxicology (Wahrheit D. B. ed.), London, Academic Press, pp. 229–257.

Fubini B. and Mollo L. (1995), Role of iron in the reactivity of mineral fibres. Toxicol. Letters **82/83**, 951–960.

Ghio A. J., Zhang J. and Piantadosi C. A. (1992), Generation of hydroxyl radical by crocidolite asbestos is proportional to surface [Fe^{3+}]. Arch. Biochem. Biophys .**298** (2), 646–650.

Ghio A. J. and Hatch G. E. (1993), Lavage phospholipid concentration after silica instillation in the rat is associated with complexed [Fe^{3+}] on the dust surface. Am. J. Respir. Cell Mol. Biol. **8**, 403–407.

Ghio A. J., Kennedy T. P., Stonehuerner J. G., Crumbliss A. L. and Hoidal J. R. (1994a), DNA strand breaks following *in vitro* exposure to asbestos increase with surface-complexed [Fe^{3+}]. Arch. Biochem. Biophys. **311** (1), 13–18.

Ghio A. J., Stonehuerner J. G., Steele M. P. and Crumbliss A. L. (1994b), Phagocyte-generated superoxide reduces Fe^{3+} to displace it from the surface of asbestos. Arch. Biochem. Biophys. **315** (2), 219–225.

Glass L. R., Mast R. W., Hesterberg T. H., Anderson R., McConnell E. E. and Bernstein D. M. (1992), Inhalation oncogenicity study of kaolin refractory ceramic fibre (RCF) in rats – final results. Toxicologist **12**, 377.

Greim H. (1993), Gesundheitsschädliche Arbeitsstoffe: Toxikologisch – arbeitsmedizinische Begründung von MAK-Werten, Faserstäube. 19. Lieferung, VCH Verlagsgesellschaft, Weinheim.

Hammad Y., Simmons W., Abdel-Kder H., Reynolds C. and Weill H. (1988), Effect of chemical composition on pulmonary clearance of man-made mineral fibres. Ann. Occup. Hyg. **32**, 769–779.

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

Hesterberg T. W., Mast R., McConnell E. E., Chevalier J., Bernstein D. M., Burn W. B. and Anderson R. (1992), Chronic inhalation toxicity of refractory ceramic fibers in Syrian hamsters. In: Mechanisms of Fibre Carcinogenesis (Brown R. C., Hoskins J. A., Johnson N. eds.). NATO ASI Series A. Life Sciences 233, New York, Plenum Press, pp. 519–539.

Hesterberg T. W., Miller W. C., McConnell E. E., Chevalier J., Hadley J. G., Bernstein D. M., Thevenaz P. and Anderson R. (1993), Chronic inhalation toxicity of size seperated glass fibres in Fischer 344 rats. Fundam. Appl. Toxicol. **20**, 464–476.

Hesterberg T. W., Chase G., Axten C., Miller W. C., Musselman R. P., Kamstrup O., Hadley J., Morscheidt C., Bernstein D. M. and Thevenaz P. (1998), Biopersistence of synthetic vitreous fibers and amosite asbestos in the rat lung following inhalation. Toxicol. Appl. Pharmacol. **151**, 262–275.

Hippeli S., Dornisch K., Kaiser S., Dräger U. and Elstner E. F. (1997), Biological durability and oxidative potential of a stone wool mineral fibre compared to crocidolite asbestos fibres. Arch. Toxicol. **71**, 532–535.

Kamp D. W., Graceffa P., Prior W. A. and Weitzman S. A. (1992), The role of free radicals in asbestos induced diseases. Free Rad. Biol. Med. **12**, 293–315.

Kamp D. W. and Weitzman S. A. (1997) Asbestosis: clinical spectrum and pathogenic mechanisms. P. S. E.B. M. **214**, 12–26.

Kamstrup O., Davis J. G. M., Ellehauge A. and Guldberg M. (1998), The biopersistence and pathogenicity of man-made vitreous fibres after short- and long-term inhalation. Ann. Occup. Hyg. **42**(3), 191–199.

v. Kruedener S., Schempp H. and Elstner E. F. (1995), Gas chromatographic differentiation between myeloperoxidase activity and fenton-type oxidants. Free Rad. Biol. Med. **19**, 141–146.

Lindgren F., Sjostrom M., Berglind R. and Nyberg B. (1996), Modelling of the biological activity for a set of ceramic fibre materials: a QSAR study. SAR-QSAR-Environ. Res. **5**(4), 299–310.

Lund L. G. and Aust A. E. (1990), Iron mobilisation from asbestos by chelators and ascorbic acid. Arch. Biochem. Biophys. **278**(1), 60–64.

Martra G., Chiardola E., Coluccia S., Marchese L., Tomatis M. and Fubini B. (1999), Reactive sites at the surface of crocidolite asbestos. Langmuir. **15**, 5742–5752.

Mast R. W., Hesterberg T. W., Glass L. R., McConnell E. E., Anderson R. and Bernstein D. M. (1994), Chronic inhalation and biopersistence of refractory ceramic fibre in rats and hamsters. Environ. Health Perspect. **102**(Suppl 5), 207–209.

McConnell E. E., Kamstrup O., Musselman R., Hesterberg T. W., Chevalier J., Miller W. C. and Thevenaz P. (1994), Chronic inhalation study of size separated rock and slag wool insulation fibers in Fischer 344/N rats. Inhal. Toxicol. **6**(6), 571–614.

Meringo de A., Morscheidt C., Thélohan S. and Tiesler H. (1994), *In vitro* assessment of biodurability: acellular systems. Environ. Health Perspect. **102** (Suppl. 5), 47–53.

Morgan M. and Holmes A. (1984), Solubility of rock wool *in vivo* and the formation of pseudo-asbestos bodies. Ann. Occup. Hyg. **28**, 307–314.

Mossman B. T. and Landesman J. M. (1983), Importance of oxygen free radicals in asbestos-induced injury to airway epithelial cells. Chest **83**, 503–515.

Muhle H., Bellmann B., Sebastian K., Böhm T., Nies E. and Barig A. (1998), BIA-Report 2/98 Fasern – Tests zur Abschätzung der Biobeständigkeit und zum Verstaubungsverhalten. Hauptverband der gewerblichen Berufsgenossenschaften (HVBG) (ed.), Alte Heerstr. 111, 53754 Sankt Augustin, pp. 369.

Musselman R. P., Miller W. C., Eastes W., Hadley J. G., Kamstrup O., Thevenaz P. and Hesterberg T. W. (1994), Biopersistences of man-made vitreous fibres and crocidolite fibres in rat lungs following short-term exposures. Environ. Health Perspect. **102**(Suppl. 5), 139–143.

Respondek M., Wiethege Th. and Müller K.-M. (1995), Pulmonale Reaktionsmuster auf künstliche Mineralfasern, Forschungsbericht FZKA-PUG 21, (Projekt Umwelt und Gesundheit ed.), Forschungszentrum Karlsruhe, Postfach 3640, 76021 Karlsruhe, Germany, pp. 74.

Roggli V. L., George M. and Brody A. R. (1987), Clearance and dimensional changes of crocidolite asbestos fibers isolated from lungs of rats following short-term exposure. Environ. Res. **42**, 94–105.

Scholze H. and Conradt R. (1987), An *in vitro* study of the chemical durability of siliceous fibres. Ann. Occup. Hyg. **31**(4B), 683–692.

Stanton M. and Wrench C. (1972), Mechanisms of mesothelioma induction with asbestos and fibrous glass. J. Natl. Cancer Inst. **48**, 797–820.

Timbrell V. and Rendall R. E. G. (1971/72), Preparation of the UICC standard reference samples of asbestos. Powder Technol. **5**, 279–287.

Weitzman S. A. and Graceffa P. (1984), Asbestos catalyses hydroxyl and superoxide radical generation from hydrogen peroxide. Arch. Biochem. Biophys. **288**, 373–376.

Wilson R., Langer A. M. and Nolan R. P. (1999), A risk assessment for exposure to glass wool. Regul. Toxicol. Pharmacol. **30**, 96–109.

Zalma R., Guignard J., Pezerat H. and Jaurand M. C. (1989), Production of radicals arising from surface activity of fibrous minerals. In: Effects of Mineral Dusts on Cells (Mossman B. T. and Begin R. eds.), NATO ASI Series H, Springer Verlag Berlin, Vol. 30, pp. 257–264.