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The release of iron from different asbestos structures by hydrogen peroxide with concomitant O_2 generation

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Treatment of aqueous suspensions of different asbestos fibers (amosite, anthophyllite, chrysotile, and crocidolite) at 0–4°C and pH 7.2 with H_2O_2 results in the consumption of H_2O_2 with concomitant release of iron and production of O_2. During incubations, [H_2O_2] decreased in proportion to the mass of the suspended fiber, the duration of incubation, and the initial [H_2O_2]. The consumption of H_2O_2, production of O_2 and release of iron all vary synergistically with the structure of the asbestos fiber. Release of silicon during the incubation was small relative to the decrement in [H_2O_2], reflecting a lack of dissolution of the fiber. The data are consistent with a redox process for the release of surface bound iron and it is significant that iron release occurs in the absence of a Fe(II) or Fe(III) chelator. The implications of iron release from the asbestos surface may be important in inflammatory disorders in which both silicate bound iron and H_2O_2 accumulate.

Keywords: asbestos, hydrogen peroxide, iron, oxidation-reduction

Introduction

Inhalation of asbestos can cause pleural disease, pneumoconiosis, and lung carcinoma in humans. Some portion of lung injury, morbidity, and mortality after exposure to these fibrous silicates results from the generation of oxygen-based free radicals by the particle (Mossman *et al.* 1990, Kamp, *et al.* 1992, Hardy & Aust 1995, Ghio *et al.* 1997), including O_2^- (Nyberg & Klockars 1990, Takeuchi *et al.* 1993), H_2O_2 (Leanderson & Tagesson 1992, Takeuchi *et al.* 1993), and ·OH (Weitzman & Graceffa 1984). These fibrous silicates are known to serve as solid phase chelants for Fe(III) through surface silanol groups and as such can serve as sites for localized high concentrations of Fe in the lung. Some portion of oxidant production by asbestos is catalyzed by Fe, either within the structural lattice of the silicate (Lund & Aust 1992) or complexed to the particle surface (Ghio *et al.* 1992a). Different asbestos materials are characterized by dissimilar structures with varying amounts of Fe(II) and Fe(III) as part of their lattice architecture, including fibers with no Fe in the ideal molecular formula (e.g. chrysotile). A second intermediate product in the reductive metabolism of O_2, H_2O_2, is also widely found in biological systems. Exposures to H_2O_2 can be associated with an injury to a biological system.

In previous studies we have shown that surface Si–OH groups on silicates bind Fe(III) from both inorganic and biological sources (Ghio *et al.* 1992a). These silicates were found to catalyze oxidant generation in a H_2O_2/ascorbate system *in vitro*, trigger respiratory burst activity and leukotriene B4 release by alveolar macrophages, and induce acute lung inflammation in the rat (Ghio *et al.* 1992a, Ghio *et al.* 1992b). Surface bound Fe(III) will cause DNA strand breaks verifying genotoxicity of silicate fibers which is related to Fe complexed to the surface. The

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DNA damage caused by silicate surface complexed Fe is presumably caused by catalysis of the generation of oxidants (Ghio *et al.* 1994a). The redox potential of Fe bound to the surface of asbestos fibers is not known. However, the redox chemistry of this complexed Fe is of biological significance with respect to its ability to catalyze the generation of oxygen-based free radicals and its ability to be displaced from the surface binding sites. In previous related studies, we found that superoxide generated by phagocytes displaces Fe from the surface of crocidolite, presumably by a reductive process (Ghio *et al.* 1994b). Removal of surface Fe decreases the potential for oxidative stress and injury to a living system after exposure to these dusts (Ghio *et al.* 1994b). In this paper, we report on the reaction of H_2O_2 with suspensions of silicate fibers containing varying amounts of structural (lattice) and surface bound iron.

Materials and methods

Fibrous silicates were obtained from Duke Scientific Corporation (Palo Alto, CA) and included UICC amosite, anthophyllite, chrysotile, and crocidolite. The silica employed was minusil-5 (Pennsylvania Glass and Sand, Pittsburgh, PA). Iron oxides (FeO, Fe_2O_3, and Fe_3O_4) were purchased from Johnson Matthey (Royston, Herts, England). Aluminum oxide (Al_2O_3) was obtained from ECC International (Cornwall, England) while magnesium oxide (MgO) was from Sigma (St. Louis, MO). All other materials were obtained from Sigma unless otherwise specified.

Decreases in [H_2O_2] after exposure to fibrous silicates

Fibrous silicates were suspended in 0.01 M sodium HEPES at pH 7.2, and aliquots of these suspensions were mixed with solutions of hydrogen peroxide in this same buffer to a total volume of 5.0 ml. The final concentration of H_2O_2 varied between 0.0 to 1000.0 μM while the mass of fibrous silicate included in the reaction mixtures ranged 0.0–20.0 mg. Samples were tightly capped in screw-cap tubes and put on a rotating wheel at 0 to 4° along with controls containing the same concentrations of peroxide in buffer with no fibers. At the end of the indicated time, samples and controls were centrifuged for 15 min at 4°C at 1500**g**. The supernatants were analyzed in triplicate for hydrogen peroxide by measuring the absorbance at 350 nm after reaction with potassium iodide and ammonium molybdate in phthalate buffer (Allen *et al.* 1952). Data are presented as the decrement in [H_2O_2] after subtracting decreases in the concentration of hydrogen peroxide occurring in the control reaction mixtures with no added fibers.

Oxygen production after exposure of H_2O_2 to fibrous silicates

Oxygen production in reaction mixtures containing both fibers and hydrogen peroxide was determined using polarographic methods. Measurements were made at 30°C in two 2.4 ml water-jacketed glass reaction chambers equipped with miniature Clark-type oxygen electrodes (Model 730, Diamond General, Ann Arbor, MI). The O_2 electrodes were calibrated at room air assuming the oxygen content of air-saturated deionized water to be 230 nmole O_2m l^{-1} under the conditions of the experiment. Pure nitrogen gas was employed to zero each electrode and to maintain a constant anoxic environment. After assuring that no O_2 current was generated by asbestos fibers (5.0 mg samples) in 0.01 M sodium HEPES at pH 7.2, the reaction was initiated by the addition of freshly prepared H_2O_2 to a final concentration of 10.0 μM. Changes in O_2 content were plotted on a Fisher Recordall chart recorder (series 5000). The O_2 production rate was determined from the slope of the recorded tracing over the first 2–3 min of the reaction. The contribution of dissolved oxygen present in the reagent was measured after the addition of H_2O_2 to nitrogen-purged chambers in the absence of asbestos. This value was subtracted from the O_2 production calculated for each sample in order to reflect the actual O_2 generation by the fibers in the presence of H_2O_2. All samples were assayed on three separate occasions with matching H_2O_2 control measurements. Data are presented as nmole O_2/min/mg fiber.

Release of iron from fibrous silicates after exposure to H_2O_2

Ten mg of fibrous silicate was suspended in 5.0 ml of 0.01 M sodium HEPES (pH 7.2) with 10.0 μM H_2O_2 for 24 hr at 0–4°C. Reaction mixtures were centrifuged for 15 min at 1500**g**. Iron concentrations in the supernatants, and standards prepared in the same buffer, were measured employing inductively coupled plasma emission spectroscopy (ICPES) (Model P40, Perkin Elmer, Norwalk, CT). The limit of detection was approximately 200 nM.

To evaluate the relationship of Fe release with increasing concentrations of hydrogen peroxide, 10.0 mg crocidolite was suspended in 5.0 ml of 0.01 M sodium HEPES (pH 7.2) with either 0.0, 10.0, 100.0, or 1000.0 μM H_2O_2 for 24 hr at 0–4 °C. Reaction mixtures were centrifuged and Fe concentrations in the supernatants were measured employing ICPES

Decrease in [H_2O_2] after exposure to mineral oxides

Ten mg of either SiO_2, FeO, Fe_2O_3, Fe_3O_4, Al_2O_3, or MgO was incubated in 0.01 M sodium HEPES at pH 7.2. Aliquots of these suspensions were mixed with 10.0 μM H_2O_2 in the same buffer (total volume of 5.0 ml). Samples were put on a rotating wheel at 0–4 °C along with controls containing the same concentrations of hydrogen peroxide

in buffer without dust. After 24 h, samples and controls were centrifuged for 15 min at 4 °C at 1500**g**. The supernatants were analyzed in triplicate for hydrogen peroxide by measuring the absorbance at 350 nm after reaction with potassium iodide and ammonium molybdate in phthalate buffer (Allen *et al.* 1952). Data are presented as the decrement in $[H_2O_2]$ after subtracting decreases in the concentration of hydrogen peroxide occurring in the control reaction mixtures with no added dust.

Release of silicon after exposure of fibrous silicates to H_2O_2

One mg of fibrous silicate was suspended in 5.0 ml of 0.01 M sodium HEPES (pH 7.2) with 1.0 mM H_2O_2 for 120 h at 0–4°C. The reaction mixture was centrifuged for 15 min at 1500**g**. Silicon in both the supernatant and standards was measured in triplicate employing a colorimetric assay (Taras *et al.* 1971). The limit of detection was 0.1 p.p.m.

Generation of hydroxyl radical by fibrous silicates after exposure to H_2O_2

An assay for hydroxylation products of salicylate was used to measure hydroxyl radical generation by fibrous silicates incubated with hydrogen peroxide (Floyd *et al.* 1985). Suspensions of 10.0 μM salicylic acid, 1.0 mM H_2O_2, and 10.0 mg fiber in 0.01 M sodium HEPES (pH 7.2) were incubated at 0–4 °C for 24 h. The supernatant was centrifuged (Beck Microfuge E) through a 0.22 μM microfuge tube filter (PGC Scientific No. 352-118) at 15 000**g**. A 100 μl sample of the eluate was injected onto a C18RP HPLC column (250 × 4.6 mm, Beckman No 235329). Salicylic acid reacts with ·OH to yield 2,3- and 2,5-dihydroxybenzoic acid. These hydroxylated products of salicylate were separate isocratically using 1.8% acetic acid, 1.5% methanol, and 30 mM citric acid. The salicylate products were quantified using a Coulochem electrochemical detector (ESA Model 5100A) set at a reducing potential of –0.40 V. The guard cell (used as a screen) was set at an oxidizing potential of +0.40 V. Measurements were done in duplicate.

Table 1. Surface area of fibrous silicates

	Molecular formula	Surface area (m^2/g)
Amosite	$(Fe^{2+},Mg)_7[Si_8O_{22}](OH)_2$	5.7 ± 0.3
Anthophyllite	$(Mg,Fe^{2+})_7[Si_8O_{22}](OH,F)_2$	11.8 ± 1.0
Chrysotile	$Mg_3[Si_2O_5](OH)_4$	26.8 ± 0.7
Crocidolite	$Na_2(Fe^{2+})_3(Fe^{3+})_2[Si_80_{22}](OH)_2$	8.3 ± 0.5

Table 2. Concentration and time dependence of decrements in $[H_2O_2]$ after incubations with crocidolite at 0–4 °C. Samples contained 1.0 mg crocidolite and 5.0 ml of 10.0 mM sodium HEPES, pH 7.2, with the indicated concentrations of H_2O_2

	Initial $[H_2O_2]$		
Time (h)	10.0 μM H_2O_2	100.0 μM H_2O_2	1000.0 μM H_2O_2
6	0.8 ± 0.0	2.0 ± 0.1	10.0 ± 0.7
24	1.0 ± 0.1	3.0 ± 0.4	12.0 ± 1.2
120	1.6 ± 0.1	5.0 ± 0.1	31.0 ± 0.4

Table 3. Silicon concentrations associated with 120 h incubation with asbestos at 0–4 °C. Samples contained 1.0 mg asbestos and 5.0 ml of 10.0 mM sodium HEPES, pH 7.2 with or without 1.0 mM H_2O_2

	μmoles silicon/g dust	
	Incubations without H_2O_2	Incubations with H_2O_2
Amosite	5 ± 1	4 ± 2
Anthophyllite	13 ± 1	14 ± 1
Chrysotile	65 ± 2	70 ± 0
Crocidolite	11 ± 2	12 ± 1

Statistics

Data are expressed as mean values ± standard deviations. Differences between multiple groups were compared using analysis of variance (Colton 1974). The *post-hoc* test used was Scheffe's. Significance was assumed at $P < 0.5$.

Results

Table 1 lists the fibrous silicates investigated along with their structures and surface areas. Surface area determinations, provided by UICC as Bruneur-Emmett-Teller nitrogen adsorption isotherms, demonstrate the serpentine chrysotile has a significantly greater value relative to the three amphibole minerals. Suspensions of 10 mg samples of the fibrous silicates amosite, anthophyllite, chrysotile, and crocidolite were exposed to H_2O_2 at 0–4 °C and a pH value of 7.2. H_2O_2 consumption was observed to occur and monitored at various time intervals. Decreases in H_2O_2 consumption varied with fibrous silicate structure as shown in Figure 1, with the highest consumption per mg observed for chrysotile. H_2O_2 consumption after a fixed time interval was found to increase with increasing amounts of suspended asbestos materials (Figure 2) and increasing concentrations of H_2O_2 (Table 2).

Concomitant with H_2O_2 consumption, O_2 production was observed for the various fibrous silicate suspensions. These results are presented as rates of O_2 production for the various asbestos structures in Figure 3. The highest O_2 production rate was observed for chrysotile.

Suspensions of the various fibrous silicates in water 0–4 °C and a pH value of 7.2 did not release an appreciable amount of Fe. However, the reaction of suspensions of the various fibrous silicates with H_2O_2 resulted in the release of significant amounts of chelatable Fe to the aqueous solution. Figure 4 illustrates these results and shows that the amount of Fe released varies with the asbestos structure. Iron release was the greatest for the chrysotile structure. Comparison of data presented in Figures 1 and 4 shows that variation in the relative amounts of H_2O_2 consumed with different asbestos structures parallels the relative amounts of Fe released. Additional evidence for the connection between H_2O_2 consumed and Fe released from the silicate fibers is illustrated in Figure 5 which shows that after a fixed time interval the amount of Fe released increases with increasing $[H_2O_2]$. Variation of the decrease in $[H_2O_2]$ with the mass of crocidolite and the initial concentration of hydrogen peroxide indicates that the chemical reaction with the fibrous silicate was limited by the availability of both the fiber and the hydrogen peroxide.

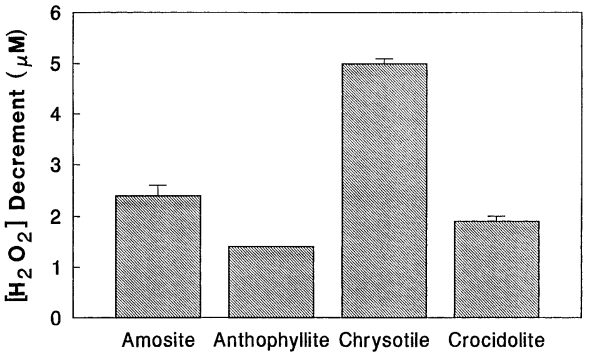

Figure 1. Decrements in $[H_2O_2]$ after one hour incubations of 10.0 mg fibrous silicates in 5.0 ml reaction mixtures containing 10.0 μM hydrogen peroxide. Decrements in $[H_2O_2]$ after fiber exposure were significantly different from the control value which was defined as zero.

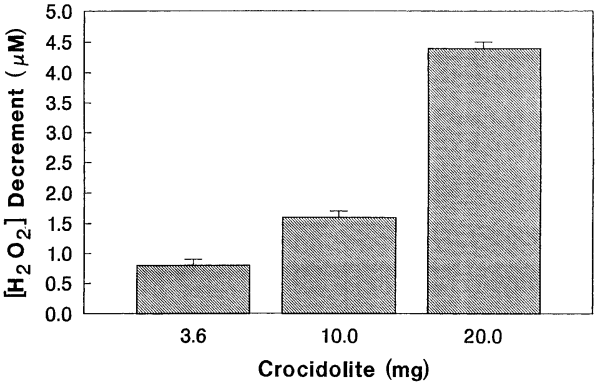

Figure 2. Decrements in $[H_2O_2]$ after one hour incubations of 5.0 ml reaction mixtures containing crocidolite in a mass varying from 0.0 to 20.0 mg. Decrements in $[H_2O_2]$ after fiber exposure were significantly different from the control value which was defined as zero.

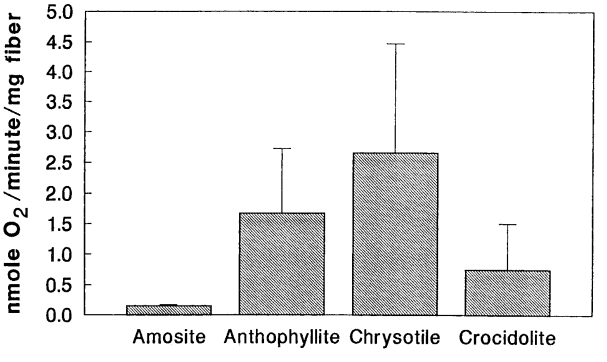

Figure 3. Oxygen production in reaction mixtures containing four fibrous silicates. There were significant differences between values of O_2 generation and the control value defined as zero.

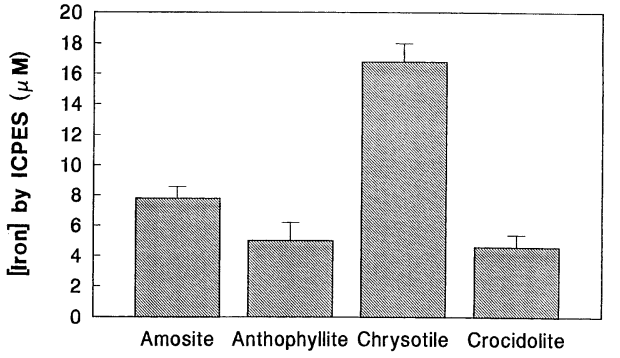

Figure 4. Iron concentrations in buffer after 24 h incubation of 10.0 mg fibrous silicates in 5.0 ml reaction mixtures containing 10.0 μM hydrogen peroxide. All iron concentrations were significantly different from the control value defined as zero.

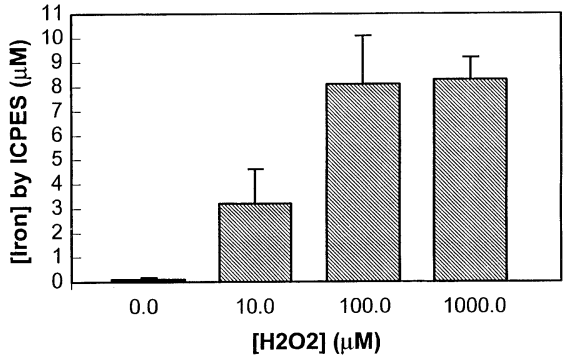

Figure 5. Iron concentrations in buffer after 24 h incubation of 10.0 mg crocidolite in 5.0 ml reaction mixtures containing 0.0 to 1000.0 μM hydrogen peroxide. There were significant differences among the groups with released [Fe] increasing with the concentration of H_2O_2 included in the mixture.

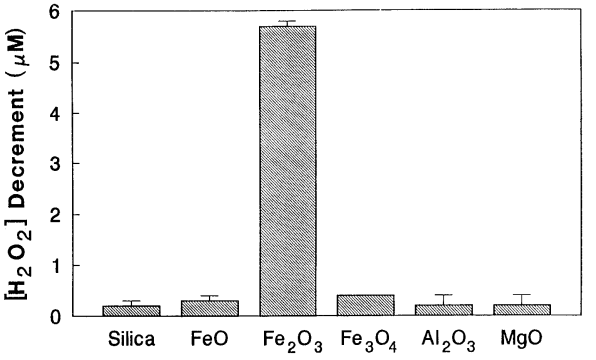

Figure 6. Decrements in $[H_2O_2]$ after 24 h incubation of mineral oxides in 5.0 ml reaction mixtures containing 10.0 μM hydrogen peroxide. Only the decrement in $[H_2O_2]$ after incubation with Fe_2O_3 was significantly different from the control value which was defined as zero.

H_2O_2 had no effect on the small amount of Si released during 120 h incubation times. This is illustrated by the data presented in Table 3 which shows identical Si release for fibrous silicate suspensions in the presence and absence of H_2O_2. Silicon release varied with increasing surface area of the silicate (Table 1).

To evaluate possible reduction of lattice cations as a source of released Fe, 10.0 mg of SiO_2, FeO, Fe_2O_3, Fe_3O_4, Al_2O_3, and MgO were incubated with 10.0 μM H_2O_2 for 24 h. Significant decrements in $[H_2O_2]$ occurred only after exposures to Fe_2O_3 (Figure 6).

Discussion

Our results demonstrate that treatment of fibrous silicates with H_2O_2 releases Fe with concomitant consumption of H_2O_2 and production of O_2. Evidence suggesting a redox process is indirect, but strongly supportive. Iron released from the silicate fibers was detected by ICPE spectroscopy. This is a sensitive technique which cannot distinguish between the oxidation states of Fe. A more direct method for detection of Fe(II) would be a chelating agent, but these agents suffer from also acting as reducing agents (Monsted and Nord 1991, Pehkonen 1995). In addition, possibly as a result of the charge of the molecules, these chelators are absorbed onto the surface of the silicates and consequently concentrations of Fe released in their presence cannot be measured reliably. The correspondence between O_2 production and H_2O_2 consumption supports H_2O_2 acting as a reducing agent and, in the process, being oxidized to O_2. The H_2O_2 driven release of Fe is therefore likely to be a reductive process.

H_2O_2 can function as an oxidizing agent, oxidizing surface Fe (as Fe^{2+} or Fe^{3+}) to ferryl 'FeO^{2+}', which is then reduced by H_2O_2 in an overall H_2O_2 disproportionation reaction. That H_2O_2 acting as an oxidizing agent to produce hydroxyl radical cannot be completely discounted, since the HEPES buffer used in these studies has been shown to scavenge hydroxyl radicals (Antholine *et al.* 1991, Grady *et al.* 1988, Hicks & Gebicki 1986, Tadolini 1987). However, the correspondence between H_2O_2 consumption and O_2 production clearly illustrates that oxidation of H_2O_2 is coupled to Fe release, which implies reduction of some Fe species bound to the silicate. It is significant that the presence of a chelator is not required for this Fe release.

Reduction of the metal, rather than oxidation, is also consistent with Fe being complexed to the surface in the +3 oxidation state. Metals at the surface of a mineral oxide have been previously established to be in a higher valence state as a result of an oxygen containing atmosphere (White & Yess 1985). This is in keeping with the hard Fe(III) ion being bound by hard surface silanol groups. Dissociation of surface-bound Fe after incubation with H_2O_2 is also consistent with the expected increased lability of Fe(II) relative to Fe(III) (Wilkins 1991).

Further evidence for Fe reduction prior to Fe release comes from the observation that only an Fe(III) oxide (Fe_2O_3), and not Fe(II) or mixed Fe(III/II) oxidation state oxides, will significantly decompose H_2O_2 at our conditions (Figure 6). This suggests that H_2O_2 decomposition by asbestos at our conditions is due to reduction of Fe(III) and not oxidation of Fe(II) on the surface or bound in the asbestos lattice.

Apparently the Fe reductively released by incubation with H_2O_2 is surface-bound since Fe release also occurs from chrysotile, which does not contain any Fe in the lattice (Table 1). Comparison of Figures 1 and 4 shows that the maximum H_2O_2 consumed and maximum Fe released is for chrysotile, which has the highest surface area of the asbestos fibers investigated (Table 1). Furthermore, incubation of the asbestos fibers with H_2O_2 does not disturb the lattice, as no increase in Si release from a suspension of asbestos fibers was observed when H_2O_2 was added (Table 3).

Our results are important in elucidating the role of asbestos exposure in human disease. Asbestos deposits in the lung can serve as solid phase chelation sites to bind Fe in high localized concentrations. Exposures to fibrous silicates are associated with an accumulation of Fe and an increase in the concentrations of H_2O_2 released by phagocytic cells (Kamp *et al.* 1994, Leanderson & Tagesson 1992). The accumulation of this metal in the immediate environment of the fiber can be recognized as a ferruginous body. Hydroxyl radical is generated after *in vivo* exposures to asbestos (Ghio *et al.* 1992b). A possible mechanism for this radical generation is the oxidation of Fe cation by H_2O_2. Our data demonstrate that, in addition to this oxidation, H_2O_2 may chemically reduce asbestos-bound Fe at physiological pH, dissociating it from the fiber surface making it available to participate in further electron transfer reactions. Significantly, our results show that mobilization of Fe from silicate fibers does not require a chelator, as previously stated (Hardy & Aust 1995).

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