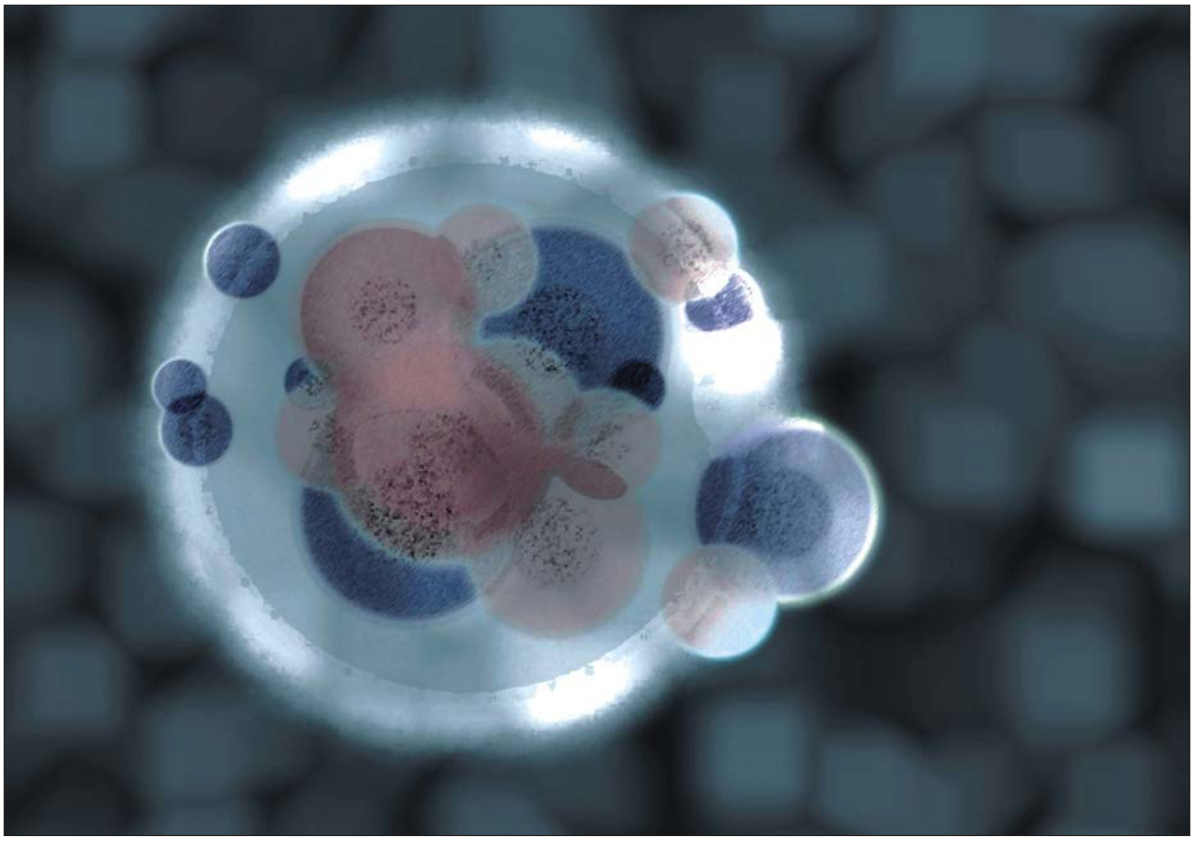

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Microdistribution of Tin in Newly Synthesized Organotin(IV)-Treated Tropical Wood Cells

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ABSTRACT
Penetration of wood preservatives into the wood cell is believed to be important to protect the wood from biodegradation especially fungal degradation. Preservatives effectiveness depends on the amount of uptake or retention as well as its uniform distribution within the wood cells. Interest on organotin(IV) complexes both mono- and disubstituted organotin(IV) is increasing due to their interesting structural features, biocidal properties and environmentally friendly. The microdistribution of tin-based preservative in tropical woods was examined using Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX) analyzer. Bulk specimens of 1% organotin(IV)-treated cubes were used to examine the microdistribution of tin in *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* woods. Conventional SEM-EDX was able to detect spatial distribution of tin in wood microstructure. The SEM-EDX distribution maps and linescan analyses showed that the deposition of tin were uneven with respect to cell microstructure in all woods studied with relatively higher tin accumulation in the ray cells and middle lamella than in the fibre cell wall. The results indicated that ray as the penetration pathway of organotin(IV) solution into the wood microstructure capable of penetrating the cell wall.

Key words: Organotin(IV) complexes, SEM-EDX analysis, microdistribution, tropical woods

INTRODUCTION

Organotin(IV) compounds are chemical compounds based on tin with hydrocarbon substituents. The chemistry of organotin(IV) compounds continues to be of interest due to their interesting structural features and also because of their potentials as agricultural biocides, antitumor agents and other biological activities which are currently being investigated by many researchers (Singh and Kaushik, 2008; Benetollo *et al*., 2005). In recent years, organotin(IV) compounds have been used extensively as agrochemical fungicides, biocides and antifouling agents (Hanif *et al*., 2010).

Various chemicals are impregnated into wood during preservative treatment. The effectiveness of preservatives depends not only upon the amount of uptake or retention, but also upon its uniform distribution in the wood cells (Zhang and Kamdem, 2000). The efficacy of preservatives is also the function of its performance in wood affected by preservative microdistribution. For instance, the excellent performance of CCA-treated softwood, particularly the *Pinus* species, is attributed to deep penetration of preservatives into the cell wall of tracheids, preservative loading, uniform preservative distribution at cell level and even preservative distribution within the cell wall (Ryan and Drysdale, 1988;

Bodner and Pekny, 1991; Newman and Murphy, 1996). In hardwoods, the poor performance of CCA is associated with failure to obtain even distribution and difficulty in achieving a desired level of chemical retention (Kamdem and Chow, 1999). Hence, when new preservatives are developed research is invariably conducted to examine their microdistribution in treated wood.

Marienfeld *et al*. (2000) used laser mass spectrometry to examine the distribution of inorganic elements in plant tissues, but electron microscopy in combination with Energy Dispersive analysis of X-rays (EDX) is the method of choice for examining the distribution and cell wall penetration of biocides, particularly metals, in preservative-treated wood. Scanning or transmission electron microscopy in combination with EDX has been used to assess whether chlorine, copper, chromium, titanium, silicon and zinc in different wood preservative systems are able to penetrate wood cell walls (Kamdem and McIntyre, 1998; Matsunaga *et al*., 2004; Cao and Kamdem, 2005; De Vetter *et al*., 2006). Some of these studies have also looked at differences in the distribution of such elements in early wood and late wood in softwoods and the vessels, fibres and rays of hardwoods (Petric *et al*., 2000; Matsunaga *et al*., 2004). Electron Induced X-ray Emission (EIXE) imaging was used to analysis of Copper Chrome Arsenic (CCA) preservatives pressure-impregnated into wood tissues by Wong *et al*. (1999). Using higher degree of analytical sensitivity a novel Particle Induced X-ray Emission (PIXE) system has also been used to analyze the distribution of copper, chromium and arsenic of CCA treated some Malaysian hardwoods (Wong *et al*., 1996). A relatively higher concentration of copper, chromium and or arsenic found in ray cells, apo and paratracheal parenchyma, vasicentric parenchyma and vessel lumina than in fibre (Wong *et al*., 1996).

Energy dispersive X-ray can be used to detect elements at a particular location in a sample (point analysis) or map the distribution of elements in a selected area. The majority of studies of the distribution of metals in preservatives-treated wood have used point analysis to detect variations in the concentrations of metal elements within cell walls and between different tissue types. Some of these studies have also included dot maps of treated woods, which show higher concentrations of metals in wood cell walls compared to lumina or in certain tissue types (Cao and Kamdem, 2005; De Vetter *et al*., 2006). Electron microscope and EDX system is easier to obtain X-ray maps at high magnifications and these have found application in the mapping of low concentrations of metals in doped nano-scale semi-conductor devices (Huang *et al*., 2005). Matsunaga *et al*. (2008) observed the metals in pine treated with copper and iron nanoparticles where used conventional and field-emission scanning-electron-microscopy and Energy-Dispersive analysis of X-rays (EDX) for treated southern pine.

Detailed X-ray microanalysis to determine the microdistribution of preservative in wood cells can be achieved with transmission electron microscope fitted with energy dispersive X-ray analysis (TEM-EDXA). The TEM-EDXA is used due to its high spatial resolution thus detail X-ray microanalytical studies on wood cell wall layer (Daniel and Nilsson, 1987) can be done and the X-ray generated outside the region of interest can be eliminated (Ryan, 1986). However, sample preparation for TEM examination is tedious and time consuming. To overcome this problem, scanning electron microscope coupled with energy dispersive X-ray analysis (SEM-EDXA) of semi thin section have shown to be useful in analyzing the microdistribution of preservative elements in wood cell walls (Matsunaga *et al*., 2004; Cao and Kamdem, 2005). For example, routine CCA microdistribution studies of large specimen areas, SEM-X-ray microanalysis of semi-thin sections provides a convenient alternative to TEM-X-ray microanalysis (Daniel and Nilsson, 1987). Jusoh and Kamdem (2009) also used SEM-EDXA to examine the microdistribution of Chromate Copper Arsenate (CCA) preservative in treated rubberwood and observed high accumulation of chromium, copper and arsenic in the vessels and lower concentration of the three preservative elements in fibres. However, the SEM-EDXA of bulk analysis is useful in evaluating the penetration pathways of preservatives in treated woods (Ryan and Drysdale, 1988).

In this present study, we reported the microdistribution of tin in the wood cells treated with synthesized organotin(IV) compounds using SEM-EDX analysis. Previous studies have shown that organotin(IV) treated wood provided protection against decay fungi (Jusoh *et al*., 2012; Rahman *et al*., 2013). The aim of this study was to determine the microdistribution pattern of tin in different wood cells of *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* following pressure treatment with three newly synthesized organotin(IV) complexes.

MATERIALS AND METHODS

Preparation and treatment of wood samples: Three non-durable tropical wood species namely *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* were selected for this study. Three newly synthesized organotin(IV) complexes (Affan *et al*., 2011) were used as wood preservatives. Mono- and diorganotin(IV) salts were purchased from Fluka, Aldrich and JT Baker. All organotin(IV) salts were used without purification to synthesize the organotin(IV) complexes. Wood treatments were carried out according to the AWPA standard E10-91 (AWPA., 1991) with slight modifications. Details of wood sample preparation and treatment of wood samples using organotin(IV) complexes have been described by Jusoh *et al*. (2012).

Scanning Electron Microscopy–Energy Dispersive X-ray Analysis (SEM-EDXA): Scanning electron microscopy in conjunction with energy dispersive X-ray analysis (SEM-EDXA) was carried out to provide information on the

distribution of tin in treated wood cells. Wood cubes treated with 1% organotin(IV) complexes were selected for SEM-EDXA. Bulk specimens of treated wood cubes were used to analyze the microdistribution of tin. The treated cubes were split into strips of 3 mm and the transverse surface were exposed using razor blade (Exley *et al*., 1974). The samples were mounted on aluminum stub using double-sided adhesive tape and sputter coated with platinum for 60 sec using JEOL JFC-1600 Auto Fine Coater and examined using a JEOL JSM-6390LA analytical scanning electron microscope (MP 14400035) fitted with Noran Vantage energy dispersive X-ray system. Operating and analyses conditions were standardized using a light element detector capable of detecting elements to atomic number 5. A 15 kV accelerating voltage with 128×96 resolution pixels, working distance 15 mm and take off angle 30^{o} were used to examine the samples. The samples were analyzed for Sn-Kα 25.27 keV and total acquisition time was 300 sec. Gross distribution pattern of tin was obtained by X-ray mapping. The map consists of bright dots and dot density is used as qualitative measure of the concentration of tin. Linescan analyses were performed on two adjacent fibre cell walls. The analysis provides information on the distribution of tin within a fibre cell wall. This study confined to the examination of tin distribution in fibre cell wall, since fibre make up the bulk of tropical wood. The X-ray intensities were measured by counting number of X-ray detected per second.

RESULTS

Microdistribution of tin in wood cell through SEM-EDXA: Scanning electron microscope in conjunction with energy dispersive X-ray analysis (SEM-EDXA) was conducted to provide information on the distribution of tin following treatment with organotin(IV) complexes. The X-ray distribution map of tin and EDX spectrum of organotin(IV) treated *Alstonia scholaris*, *Hevea brasiliensis* wood samples are shown in Fig. 1 and 2, respectively.

The X-ray maps of organotin(IV)-treated *A. scholaris* and *H. brasiliensis* wood showed that overall distribution of tin within the cells was uneven. The white dots indicate the presence of tin, where brighter dot the higher the concentration of tin (Fig. 1 and 2). The ray cell and middle lamella white dot were relatively brighter than the fibre cell wall. This suggests that organotin(IV) solution was forced into ray cells first then penetrated into the adjacent fibre cell wall. Linescan analyses on organotin(IV)-treated *Hevea brasiliensis* was carried out to detect the presence of tin within fibre cell wall. The result of a linescan analysis of *Hevea brasiliensis* wood sample treated with organotin(IV) is shown in Fig. 3. Linescan analysis showed that tin was detected in fibre cell walls but in relatively lesser quantity as compared to middle lamella (Fig. 3). The linescan showed that count rates increases from the lumen area towards the lumen surface then decreases across the S2 layer and then increases again in the middle lamella region.

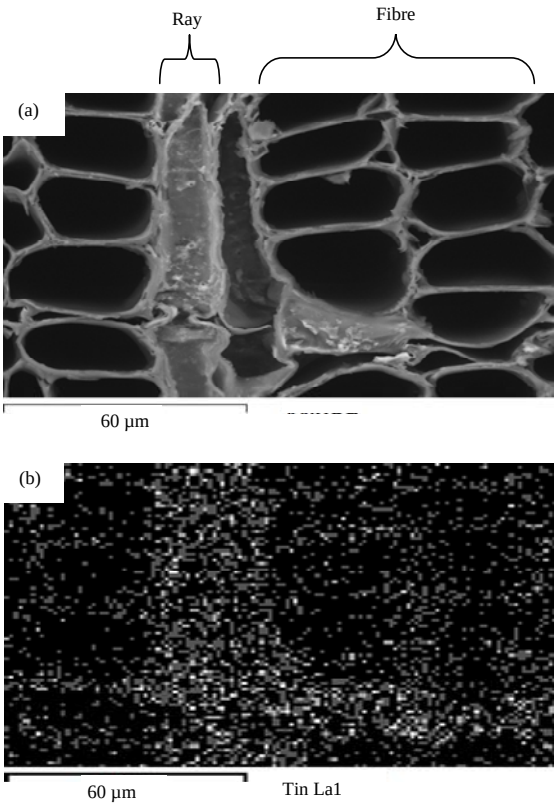

Fig. 1(a-b): (a) Transverse section of wood sample and (b) X-ray distribution map of tin in *Alstonia scholaris*

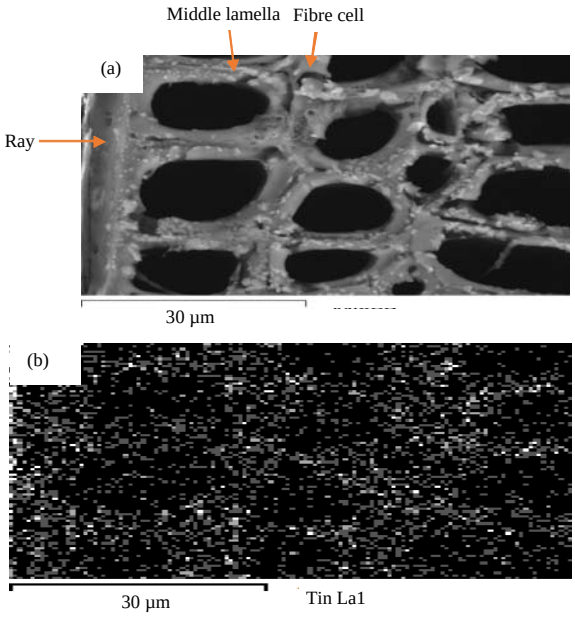

Fig. 2(a-b): (a) Transverse section of wood sample and (b) X-ray distribution map of tin in *Hevea brasiliensis*

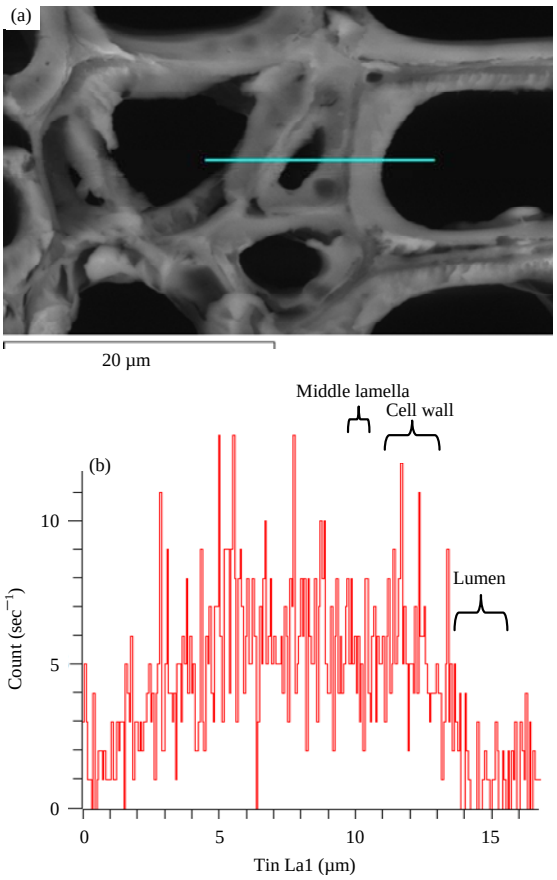

Fig. 3(a-b): SEM-EDXA linescan analysis of *Hevea brasiliensis* treated with organotin(IV) complex. (a) SEM micrograph of transverse surface showing the linescan and (b) Microdistribution of tin in fibre cell wall

DISCUSSION

Microdistribution of tin in *Alstonia scholaris, Macaranga triloba* and *Hevea brasiliensis* wood cells: Energy Dispersive X-ray Analysis (EDXA) was conducted to confirm and obtain better understanding about the incorporation of tin within wood cell. The results suggest ray cells were the major pathway of organotin(IV) solution move into the adjacent fibre cells. Ray cells as the pathways of wood preservative was also observed in *Cryptomeria japonica* (Matsunaga *et al*., 2004), *Pinu scontorta* and *Picea glauca* (An *et al*., 1998). The deposition of tin in the wood also indicates chemical bonds were formed as a result of the reaction between wood cells and tin. However, the distributions of tin in the wood cells were uneven. Accumulation of tin was variably distributed among cell microstructure. From Fig. 3, it can be observed that the X-ray intensities were lower in the S_2 cell wall layer compared to the lumen surface, S_1 cell wall layer and middle lamella. Uneven distribution of copper was detected by An *et al*. (1998) in lumen surface and ray cells of CCA-treated heartwood of lodgepole pine and white spruce. Using Field Emission Scanning Electron Microscopy (FE-SEM) in combination with X-ray microanalysis (EDX), Matsunaga *et al*. (2007) studied the southern pine wood commercially treated with Micronized Copper Quat (MCQ) and confirmed the presence of copper in the cell wall and concluded that the micro distribution of copper in MCQ treated wood looks like that observed in wood treated with amine soluble copper wood preservative. Stirling *et al*. (2008) conducted a study on MCQ treated wood using Environmental Scanning Electron Microscopy (ESEM) equipped with Energy Dispersive X-Ray Spectrometry (EDS) and their study revealed that there was a high amount of copper in the lumens and small amount of copper in cell walls of wood for both MCQ treated wood and Ammine Copper Quat (ACQ) treated wood. Higher deposition of preservative in the lumen surface and middle lamella was also reported in various softwood and hardwood species (Jusoh and Kamdem, 2009; Cao and Kamdem, 2005; Matsunaga *et al*., 2004; Newman and Murphy, 1996; Drysdale *et al*., 1980). Doyle and Ruddick (1994) studied the distribution of an alkylammonium compound, iodobenzalkonium chloride, in ponderosa pine sapwood using scanning electron microscopy coupled with an energy dispersive X-ray analyzer (SEM-EDX) and detected the highest accumulation of iodine was in the compound middle lamella.

Tin appears to penetrate into the fibre cell walls as revealed by linescan analyses, however in relatively lower concentration (Fig. 3). Low preservative accumulation in fibre cell had previously reported (Greaves, 1974; Levy and Greaves, 1978; Greaves *et al*., 1982; Dawson-Andoh and Kamdem, 1998; Jusoh and Kamdem, 2009). The cell corner and middle lamella are rich in lignin (Fengel and Wegener, 1983; Haygreen and Bowyer, 2003). Lignin plays an important role for bonding preservatives components and it has been suggested that lignin is one of the binding sites for preservative components (Daniel and Nilsson, 1987; Ryan and Drysdale, 1988). Lee *et al*. (1992) revealed that chromium, copper and arsenic were more abundant in the compound middle lamella in wood treated with Chromate Copper Arsenate (CCA). Daniel and Nilsson (1987) reported that the relative microdistribution of CCA to follow closely that of the lignin distribution and regions showing high lignin levels showed high CCA levels and vice-versa where recorded highest CCA and lignin in the vessels, fibre and ray middle lamella cell corner regions while the lowest levels were detected in the fibre (S_2) secondary walls. Daniel and Nilsson (1987) also pointed that the lignin content and fixation of preservative components varied with wood species. Another factor influencing even CCA distribution in softwoods than hardwoods may be due to the presence of G-lignins in softwoods versus higher proportions of S-lignins in many hardwoods (Kim *et al*., 2006; Nilsson *et al*., 1988).

Additionally, the distribution of lignin within the cell wall and the lignin content of different parts of a tree are not uniform. For example, high lignin amounts are characteristic for softwood branches and compression wood (Timell, 1986), whereas the gelatinous layers of the tension wood fibres in hardwoods may be almost lacking of lignin (Novaes *et al*., 2010). Scanning Electron Microscopy (SEM) combination with Energy Dispersive X-ray Analysis (EDXA) technique confirmed on a high-resolution level that middle lamella portions contain distinctly more lignin than secondary walls in wood (Donaldson and Ryan, 1987). The variation of lignin content of different wood cell is most likely due to one important cause of uneven distribution of preservatives component of different wood cell. It is well known that the cell corner and middle lamella regions are constituents with the highest lignin content (Fengel and Wegener, 1983). Compound middle lamellae are generally rich in lignin with lignin and hemicelluloses accounting for more than 90% of this zone which may have caused the greater copper concentration in the compound middle lamella (Matsunaga *et al*., 2004). The uneven copper concentration in Japanese cedar sapwood, although the aqueous solution penetrated the sapwood specimen entirely (Matsunaga *et al*., 2004).

For any full cell preservative treatment like this study it is expected that the cell lumina are filled with preservative solution and as the wood dries the preservative components are deposited on the lumen wall (Hedley *et al*., 1990). Conventional SEM images and EDX maps of treated southern pine revealed that both copper and iron were deposited in the lumina of rays and some tracheids and also on bordered pits (Matsunaga *et al*., 2008). Dawson-Andoh and Kamdem (1998) studied copper microdistribution in copper naphthenate treated soft maple and northern red oak where used environmental scanning electron microscopy and detected high counts of copper in the vessels of both treated wood species. Jusoh and Kamdem (2009) studied the microdistribution of Chromate Copper Arsenate (CCA) preservative in rubberwood using scanning electron microscope in conjunction with energy dispersive X-ray analyzer (SEM-EDXA) and observed a high accumulation of chromium, copper and arsenic in the vessels and lower concentration of the three preservative elements in fibres. The increase of the solution strength in chromium, copper and arsenic corresponds to an increase in Cr, Cu and As level in wood cells (Jusoh and Kamdem, 2009).

CONCLUSION

Conventional SEM-EDX analysis was able to detect the distribution of tin in wood following treatment with organotin(IV) compounds. The main organotin(IV) movement apparently occurred along the ray then redistributed into the adjacent cells. The microdistribution pattern of tin was uneven with higher accumulation in the ray cell and middle lamella. X-ray mapping and line-scan analyses showed that the newly synthesized organotin(IV) compounds successfully incorporated within the wood cell. The results indicated that tropical wood species are treatable with the synthesized organotin(IV) complexes.

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