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Laboratory studies on formation of bound residues and degradation of propiconazole in soils

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Abstract: Laboratory studies on the formation of bound residues and on the degradation of the triazole fungicide propiconazole were conducted in two different soils. Soils treated with ^{14}C-propiconazole were incubated at 22 °C and extracted exhaustively with a solvent at each sampling date until no further propiconazole was extracted. The solvent-extractable residues were used to measure propiconazole remaining in the soil, and the extracted soils were used to investigate bound residues of propiconazole. Mineralization of propiconazole was investigated by measuring [^{14}C]carbon dioxide evolved from the soil samples. Formation of bound residues of propiconazole was higher in silty clay loam soil than in sandy loam soil, giving approximately 38 and 23% of the applied ^{14}C, respectively. In contrast, the rates of degradation and mineralization of propiconazole were lower in silty clay loam soil than in sandy loam soil. Decreased extractability of the ^{14}C residues with incubation time was observed with increased formation of bound residues. When the propiconazole remaining in the solvent-extractable residues was quantitatively measured by high-pressure liquid chromatographic analysis, the half-life value in sandy loam soil was about 315 days, while the half-life in silty clay loam soil exceeded the duration of the 1 year experimental period. Increased formation of bound residues was observed as propiconazole degraded with incubation time, suggesting that degradation products are involved in the formation of bound residues. Our study suggests that the formation of bound residues of propiconazole contributes to the persistence of this fungicide in soil.

Keywords: propiconazole; triazole fungicide; bound residues; soil

1 INTRODUCTION

Triazole fungicides are now widely used in agriculture because of their broad range of action at low application rates. Although these fungicides are not directly sprayed onto soil, they can reach the soil. The widespread use and repeated application of these fungicides, therefore, may inadvertently lead to their accumulation in soil. In the pesticide-accumulated soil, one of main processes that could influence pesticide degradation is sorption. Many pesticides tend to form bound residues.[1–3] Bound residues are, basically, unextractable and chemically unidentifiable pesticide residues remaining in fulvic acid, humic acid and humin fractions of soil after exhaustive solvent extraction.[4]

Bound residues are one of the most important factors that may contribute to the accumulation of triazole pesticides such as propiconazole. This pesticide has a high adsorption coefficient and low mobility in soil.[5–8] Pesticide accumulation in soil is a possible cause of environmental concern to soil ecosystems.[9–13] Fresh and clean soil environments are based on continuous cycling of soil elements by soil biota. These elements include pesticides that accumulate in soil. Pesticide persistence in soil is often believed to be a consequence of their limited biodegradation. Formation of bound residues of pesticides in soil is expected to be one of the explanations of why persistent pesticides are not easily incorporated into biogenic element cycles for degradation.

Propiconazole, 1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole, is a systemic triazole fungicide with broad spectrum of specificity against fungal pathogens.[14–16] A number of studies on the degradation of propiconazole in soil have recently appeared since this pesticide has been found to be persistent in soil.[17–20] The persistence of propiconazole in soil might be an environmental

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concern because of the risk of soil contamination given its repeated application and high accumulation in soil. Little information is known about the type of pesticide residues that involve the persistence of propiconazole in soil. The present study was performed to address this. Soils treated with propiconazole were incubated over a 1-year period. The degradation of propiconazole was investigated by characterizing bound residues, measuring mineralization and identifying degradation products.

2 MATERIALS AND METHODS

2.1 Chemicals

All chemicals used in this study were of analytical grade, unless otherwise stated. [U-*phenyl*-^{14}C]propiconazole (specific activity 808.45 MBq mmol^{-1}) was purchased from International Isotope München (Stadionstra, Germany). The radiochemical purity was confirmed to be greater than 98% by thin-layer chromatography (TLC) and autoradiography (Fuji BAS 1500, Tokyo, Japan). Propiconazole was kindly supplied by Novartis (Greensboro, NC, USA).

2.2 Soils

Two different types of soil, silty clay loam and sandy loam, were collected from the topsoil of agricultural fields located at Naju, Chonnam and Yong-Bong Dong, Kwangju, in South Korea. The soils had no past history of propiconazole treatment. Some of the physicochemical properties of these soils are given in Table 1. Soils were passed through a 4-mm sieve without drying, mixed thoroughly and stored in the dark at 4 °C until use.

2.3 Propiconazole incubation

Two kilograms of each soil were equilibrated in the dark at 22 (±1) °C for 1 week before propiconazole was added. Thirty-gram portions of each soil were weighed and transferred to a series of triplicate 250-ml flasks. [^{14}C]Propiconazole (71 kBq) dissolved in acetone was carefully added dropwise to the soil to give an initial concentration of 1.0 mg kg^{-1}. The acetone was allowed to evaporate in a ventilated hood at room temperature. Blank soils treated with acetone alone were prepared as described above. The treated soils were then thoroughly mixed and distilled water was added to give a moisture content equivalent to 60% field moisture capacity. The flasks were incubated in the dark at 22 (±1) °C over a 1-year period. The moisture content of the soil was maintained at the initial level by replenishing water loss weekly. Triplicate flasks were taken monthly or bimonthly for analysis and stored at −20 °C until analyzed.

In a concurrent experiment, soil samples for isolation of propiconazole degradation products were prepared as described above. The unlabelled propiconazole was used to avoid a possibility of radioactive contamination of gas chromatography mass spectrometer (GC/MS) when the samples were analyzed.

2.4 Solvent-extractable and bound residues of propiconazole

Soil samples were extracted with methanol + water (80 + 20 by volume, '80% aqueous methanol' hereafter) by orbital shaking for 2 h. The extracts were centrifuged at 10 000 *g* for 20 min, and the supernatant and precipitate (soils) were separately collected. The precipitate soils were extracted again as described above. This extraction was repeated until the radioactive level of supernatant in the final extract reached the background of 80% aqueous methanol. The supernatants were used to measure propiconazole remaining in the solvent-extractable residues. Total solvent-extractable radioactivity was determined by combining the supernatants and using an aliquot of total supernatant for radioactivity assay.

The extracted soil obtained above was air-dried and used to investigate bound residues as described ealier.[7] Briefly, the soil was combusted to determine total radioactivity of bound residues before characterization. The soil was extracted with sodium pyrophosphate (0.2 M) until the radioactive counting level in the final extract reached that of the sodium pyrophosphate solution. Sodium pyrophosphate non-extractable fraction was taken as humin fraction. Sodium pyrophosphate extractable fractions were acidified with concentrated hydrochloric acid until no more precipitate was observed, and centrifuged at 16 000 *g* for 20 min. The supernatant and precipitate were taken as fulvic acid and humic acid fractions, respectively. The volume of supernatant was measured, and an aliquot of supernatant was used for determining radioactivity in the fulvic acid fraction. The precipitate was dissolved again in sodium hydroxide (0.1 M) and used for determining radioactivity in the humic acid fraction.

2.5 Propiconazole degradation

Propiconazole degradation was investigated by measuring the propiconazole remaining in solvent extractable residues. The solvent-extractable residues

Table 1. Some physicochemical characteristics of the soils used

Soils	pH (1:5 in water)	OM (%)	CEC (cmol$^+$ kg^{-1})	Moisture content (%)	Soil components (%) Sand	Silt	Clay
Sandy loam	6.7	0.8	8.7	11.4	66	26	8
Silty clay loam	7.1	3.2	10.2	24.7	14	67	19

obtained above were evaporated at 50 °C using a vacuum evaporator, and the total volume remaining after evaporation was measured. The residues were then extracted twice with their volume of dichloromethane until the radioactivity found in the final extract reached that of the blank sample. After allowing the mixture to separate, the organic phase was dried over anhydrous sodium sulfate and then concentrated to a small volume (about 3 ml) in an evaporator. The remaining residue was evaporated to dryness under a gentle stream of nitrogen gas. The dried residue was then dissolved in hexane (3 ml) and subjected to column chromatography.

A chromatographic glass column (15 mm ID × 50 cm length) was slurry-packed with 10 g Florisil^R (60–100 mesh, Aldrich, St Louis, MO, USA) in hexane. The column was prewashed with hexane (100 ml), and the dissolved sample in hexane was carefully added to the column. The column was washed again with acetone + hexane (3 + 97 by volume; 100 ml) and this eluate was discarded. The column was finally washed with acetone + hexane (10 + 90 by volume; 130 ml), and this fraction was used for propiconazole analysis. The fraction was evaporated to dryness and dissolved in methanol + water (70 + 30 by volume; 2 ml). An aliquot (20 µl) was injected into a high-pressure liquid chromatography instrument (Waters 510 pump). The mobile phase for HPLC was methanol + water (70 + 30 by volume) at a flow rate of 1.2 ml min^{-1}, and the analytical column was a µ-Bondapak C-18 stainless column (3.9 mm × 300 mm length, Waters). Propiconazole was detected at a wavelength of 220 nm, and its residue was determined by comparing retention times with calibration curves of working stock solutions of propiconazole standards.

2.6 Mineralization and volatilization of propiconazole

Mineralization and volatilization of propiconazole from the soil samples were measured during the experimental period with the method as described in previous studies.[7,8] The evolved ^{14}C-volatile metabolites and [^{14}C]carbon dioxide were trapped in sulfuric acid (1 M) and a series of two sodium hydroxide (2 M) traps, respectively. Radioactivity was measured weekly as described below.

2.7 ^{14}C-radioactivity determination

Total radioactivity in soil samples was measured by first combusting 0.3 g (air-dry weight) of soil in a Model 307 Biological Sample Oxidizer (Packard, CT, USA), and the evolved [^{14}C]carbon dioxide was then trapped in a scintillation cocktail, Carbosorb E plus PermaFour E$^+$ (Packard, CT, USA). Radioactivity was determined using a Wallac 1409 liquid scintillation counter (LSC, Helsinki, Finland). For the determination of radioactivity in the samples chemically extracted and trapped in sulfuric acid and sodium hydroxide, the scintillation cocktail Ultima Gold^R (Packard, USA) was used. No quenching effects were observed from any of the chemicals used.

2.8 Isolation of degradation products of propiconazole

Soil samples were extracted with 80% aqueous methanol and evaporated as described in the previous section. Total sample volumes were measured, and the samples were extracted again twice with their volume of ethyl acetate at pH 2. The organic phase was dehydrated and concentrated to a small volume (about 3 ml) in an evaporator. The remaining ethyl acetate was dried under a gentle stream of nitrogen gas. The dried extract was purified through a series of solvent washes on a Supelco C_{18} SPE cartridge column (Bellefonte, PA, USA). The solvent mixture contained methanol and water where the concentration of methanol was increased with each wash. SPE washing fractions were concentrated under a gentle stream of nitrogen gas and then extracted with ethyl acetate as described above. The ethyl acetate extract was evaporated to dryness under a gentle stream of nitrogen gas and dissolved in acetone (100 µl). Representative aliquots were spotted onto TLC plates (silica gel 60 F_{254} 20 × 20 cm^2, 0.5 mm thickness, Merck, Germany) followed by autoradiography. The TLC developing solvent was hexane + acetone + benzene (5 + 4 + 3 by volume). To determine concentrations of degradation products, the TLC plate corresponding to the black positive spots on the autoradiogram was scraped off, placed into 15-ml LSC vials and the radioactivity was measured in Ultima Gold^R by using a liquid scintillation counter as described above.

For isolation of propiconazole degradation products, the soil samples treated with unlabelled propiconazole were used. The positions identically located to black TLC spots of ^{14}C samples were scraped off and extracted with methanol + water (70 + 30 by volume) by an ultrasonic cleaner for 30 min. Degradation products were simultaneously detected using a Flow Scintillation Analyzer (Radiometric 150TR, Packard, CT, USA) with UV/Vis detector at a wavelength of 220 nm. The fractions corresponding to peaks from the UV/Vis detector were collected and extracted with ethyl acetate at pH2 as described above. The organic phase was dehydrated and concentrated for GC/MS analysis.

2.9 GC/MS analysis

A Varian 3000× series GC/MS (Varian, CA, USA) was used for analysis of degradation products. The column was a J & W Scientific DB-5MS (30 m × 0.2 mm ID, film thickness 0.25 µm) capillary column. Operating conditions of GC/MS were as follows: injector temperature 230 °C; GC–MS interface temperature 250 °C; oven program temperature: 100 °C (5 min), increased at 10 °C min^{-1} to 260 °C; carrier gas, helium, at a constant flow 1.0 ml min^{-1} with split ratio 50:1.

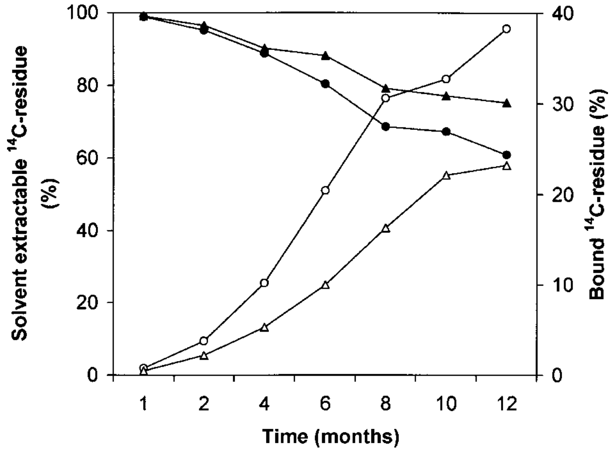

Figure 1. Formation of (●,▲) solvent-extractable and (○,△) bound residues of propiconazole in (▲,△) sandy loam and (●,○) silty clay soils.

2.10 Statistics

The data given in this study are means of three replications. The significance among data was determined using a Microsoft Excel computer program (Windows® NT). The data of three replications are not significantly different at the 5% level of standard deviation.

3 RESULTS AND DISCUSSION

3.1 Bound residues

Solvent-extractable residues of propiconazole decreased steadily with incubation time (Fig 1), while the formation of bound residues increased more and more with time. After a 1-year incubation period, approximately 60 and 74% of the applied ^{14}C was observed as solvent-extractable residues in silty clay loam and sandy loam soil, respectively. A decrease in total mass based on total radioactivity of solvent-extractable residues and losses due to mineralization was observed as the formation of bound residues increased. The decrease in solvent-extractable residues with incubation time was much higher in silty clay loam soil than in sandy loam soil, resulting in bound residues up to 38 and 23% of the applied ^{14}C, respectively. This suggests that there exists a high formation of bound residues of propiconazole in soil with high organic matter. These levels of bound residues are significantly higher than those reported in a previous study,[7] where bound residues of propiconazole were investigated in soil of a similar type under anaerobic conditions. These observations suggest the possibility that the formation of bound residues of propiconazole is much higher under aerobic conditions than under anaerobic conditions.

Most of the total bound residues in soils were found in the humin fraction (Tables 2 and 3), giving about 90% of total bound residues in that fraction. This suggests that this type of residue is the main portion of the ^{14}C found in solvent non-extractable residues. In previous studies, similar percentages of bound residues between fulvic acid and humin fractions have been reported under anaerobic conditions.[7,8] In this study, a very high percentage of bound residues was observed in the humin fraction. The fulvic acid fraction was observed to contain a high portion of bound residue in sandy loam soil. This suggests variable types of bound residues of propiconazole, depending on soil type. Increased bound residues in the fulvic fraction were also observed as the residues in the humin fraction decreased. The reason for these observations is still not clear. It should be of interest to investigate these findings in more detail to better understand the degradation behaviour of propiconazole in soil.

3.2 Mineralization and volatilization

The losses of applied [^{14}C]propiconazole by mineralization appeared to proceed slowly throughout the experiment (Fig 2). The total cumulative amount of propiconazole mineralized in sandy loam soil was approximately 8% of the applied ^{14}C. This level was higher than the 4% found in silty clay loam soil. Propiconazole has been reported to degrade biologically in soil.[21] The pattern of mineralization of propiconazole was not significantly different between soils used in this study, giving low rates of propiconazole mineralization up to $<10\%$ of the applied amounts. We hypothesized two possible reasons for the low mineralization of propiconazole in the soil: one may be the low biodegradability of the compound by soil microbial species that could degrade the fungicide or its metabolites; another possibility for low mineralization may be a strong adsorption to soil organic matter.

The rate of mineralization of propiconazole was higher in sandy loam soil than in silty clay loam soil. Silty clay loam soil contains more organic matter.

Table 2. Bound residues of [^{14}C]propiconazole in sandy loam soil

Time (months)	Total bound residues of ^{14}C (% of applied)	Relative to total bound residues (%)		
		Fulvic acid	Humic acid	Humin
1	0.5	16.9	<0.10	81.1
2	2.3	16.7	3.1	79.4
4	5.3	26.5	3.3	69.7
6	10.1	27.3	6.2	64.5
8	16.4	30.9	10.2	53.7
10	22.3	33.2	11.1	53.2
12	23.2	27.7	9.9	61.6

Table 3. Bound residues of [^{14}C]propiconazole in silty clay loam soil

Time (months)	Total bound residues of ^{14}C (% of applied)	Relative to total bound residues (%)		
		Fulvic acid	Humic acid	Humin
1	0.8	8.8	<0.10	89.1
2	3.82	7.2	0.3	88.7
4	10.3	13.9	3.6	80.7
6	20.5	10.7	8.1	79.6
8	30.6	10.3	6.4	80.6
10	32.7	12.2	3.6	81.3
12	38.3	8.3	1.5	88.4

More mineralization of propiconazole in sandy loam soil suggested, therefore, that propiconazole and its metabolites were more accessible to biological degradation. The amounts of propiconazole degraded by mineralization were similar to those found under anaerobic conditions in previous studies.[7,8] This suggests low degradation of propiconazole by a mineralization pathway. In addition to evolved [^{14}C]carbon dioxide, the background level of volatile ^{14}C exiting from the soil was observed, indicating that propiconazole was chemically stable under experimental conditions. Our results suggest that microbial mineralization and chemical volatilization are not main pathways for the disappearance of propiconazole in soil under these experimental conditions.

3.3 Degradation

Degradation of propiconazole was investigated by quantitatively measuring propiconazole found in solvent-extractable residues. The amounts of propiconazole remaining in silty clay loam and sandy loam soils after the one-year incubation time were approximately 57 and 41% of the applied ^{14}C, respectively. Propiconazole degradation was faster in sandy loam soil than in silty clay loam soil (Fig 3). Initial degradation of propiconazole was slightly higher in silty clay loam soil than in sandy loam soil, but the rate of degradation in the former decreased more and more with the incubation time. The half-life value of propiconazole in sandy loam soil was about 315 days, which was longer than that observed in previous studies.[17–19] This was probably due to the difference in extraction methods between the studies. In most studies evaluating the disappearance of pesticides in soil, pesticides are believed to extract successfully with a conventional extraction method, shaking vigorously once or twice. Propiconazole was extracted only once by shaking in previous studies.[17–19] In our study propiconazole was exhaustively extracted until none was found in the final extract. At least four or five extractions were required to reach this point.

Approximately 72% (sandy loam soil) and 60% (silty clay loam soil) of total extracted propiconazole was found in the first extract, giving 28% (sandy loam soil) and 40% (silty clay loam soil) of the total extracted propiconazole still remaining as bound residues in the soils after the first extraction. These observations suggest that the extraction efficiency of propiconazole could decrease more and more with incubation time unless the pesticide is extracted exhaustively. The half-life of propiconazole in silty clay loam soil exceeded the duration of the experiment, suggesting much slower degradation of propiconazole in this soil type. In general, much faster degradation of pesticides would be observed in soil with high organic matter contents because of the high

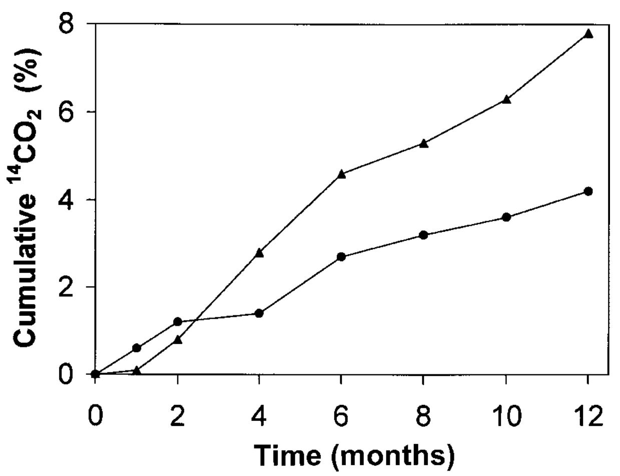

Figure 2. Cumulative percentage of [^{14}C]carbon dioxide released from (▲) sandy loam soil and (●) silty clay loam soil.

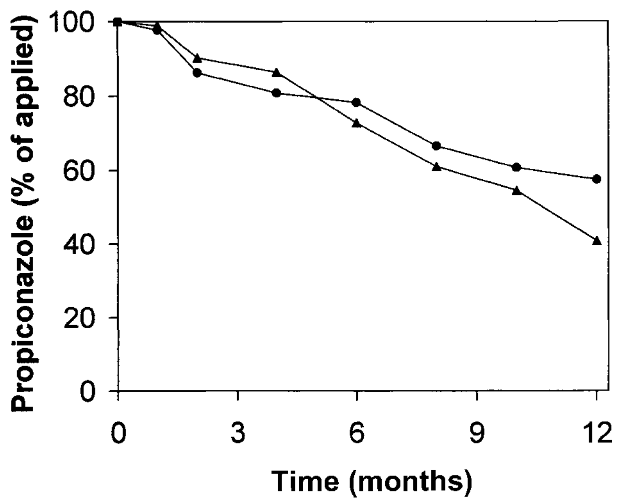

Figure 3. Degradation of propiconazole in (▲) sandy loam soil and (●) silty clay loam soil over a 1-year incubation period. The values are percentages of the applied propiconazole.

Table 4. GC/MS ion spectra of propiconazole standard and degradation products (DP) isolated from soil

		Most abundant ions	
Compound	*Mass fragments (m/z)*	*m/z*	*Abundance (%)*
Propiconazole	342, 259, 191, 109, 69	259/261/263	100/65/10
DP-1	256, 220, 173, 109, 74	173/175/177	100/70/20
DP-2	258, 222, 175, 128, 111, 82	82/84/86	100/60/20
DP-3,4	358, 342, 173, 256, 173, 109, 85	173/175/177	100/65/10

biological activity in organic-rich soil. Our results, therefore, suggest that the high formation of bound residues of propiconazole in silty clay loam soil results in less degradation of this pesticide in the soil.

3.4 Identification of degradation products

The degradation product DP-1 of propiconazole had *m/z* of 256, 220, 173 (base peak), 109 and 74 as a GC/MS fragment ion spectrum (Table 4). The molecular ion spectrum of *m/z* 256 suggests that DP-1 is 2,4-dichlorophenyl 1,2,4-triazole-1-ylmethyl ketone resulting from the degradation of the dioxolane ring in propiconazole. DP-2 was identified by its molecular ion peak at *m/z* 258 (Table 4). The fragment ion spectra of DP-2 were *m/z* 258, 222, 175, 128 and 82. On the basis of the pattern of mass fragments and comparing them to those of propiconazole standards, DP-2 was identified as 1-(2,4-dichlorophenyl)-2-(1,2,4-triazole-1-yl)ethanol. DP-3 and DP-4 showed the same mass patterns of *m/z* 358, 342, 256, 173 (base peak) and 85 as fragment ion spectra (Table 4). The fragment ion spectrum of *m/z* 342 was identified by its $(M-16)^+$, which was due to loss of an OH from *m/z* 358, suggesting that DP-3 and DP-4 are 1-[2-(2,4-dichlorophenyl)-4-(hydroxypropyl)-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole. The same patterns of mass fragmentation between DP-3 and DP-4 suggest that the molecular structures of DP-3 and DP-4 are very similar, but not exactly the same. Propiconazole has a propyl side-chain on the dioxolane ring. Therefore, these degradation products were suggested to be compounds hydroxylated on different carbon position of the propyl side-chain. Much higher concentrations of degradation products were observed in sandy loam soil than in silty clay loam soil, suggesting more degradation of propiconazole in the former. These results are reasonable with findings of a lower rate of degradation and a higher rate of bound residue formation in silty clay loam soil than in sandy loam soil.

In conclusion, our results are consistent with previous observations[17–20] that propiconazole is persistent in soil, and show for the first time that the persistence of propiconazole in soil is possibly due to a high formation of bound residues, and that these residues are more likely to be formed in high organic soil.

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