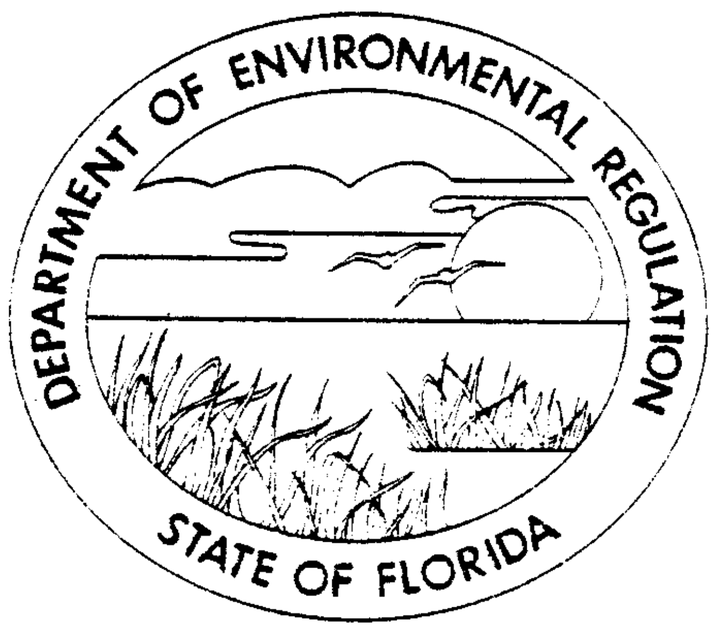


BIOASSAYS OF CARROLLWOOD VILLAGE

REGIONAL WASTEWATER TREATMENT PLANT

TAMPA, HILLSBOROUGH COUNTY, FLORIDA

SAMPLED 8/5/80

Biological Section
Bureau of Water Analysis
December 22, 1980

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INTRODUCTION

The Carrollwood Village Regional Wastewater Treatment Plant is a relatively small plant serving a residential section northwest of Tampa, Hillsborough County, Florida (Figure 1.). Treatment of sewage consists of aeration by an oxidation ditch system, clarification, tertiary filtration, final clarification, and retention in a polishing pond. Part of the effluent is then discharged to Brushy Creek, which flows to Rocky Creek hence to Old Tampa Bay, while the remainder is used for spray irrigation of several golf courses.

Because this plant is not an NPDES permitted facility, the Hillsborough County Environmental Protection Commission requested bioassay tests be performed on the effluent to determine its potential toxicity. On 6 August 1980, the Biological Section obtained effluent samples from the Carrrollwood Village Regional Wastewater Treatment Plant and performed static bioassays to determine the existence and extent of toxicity to the biota of the receiving waters.

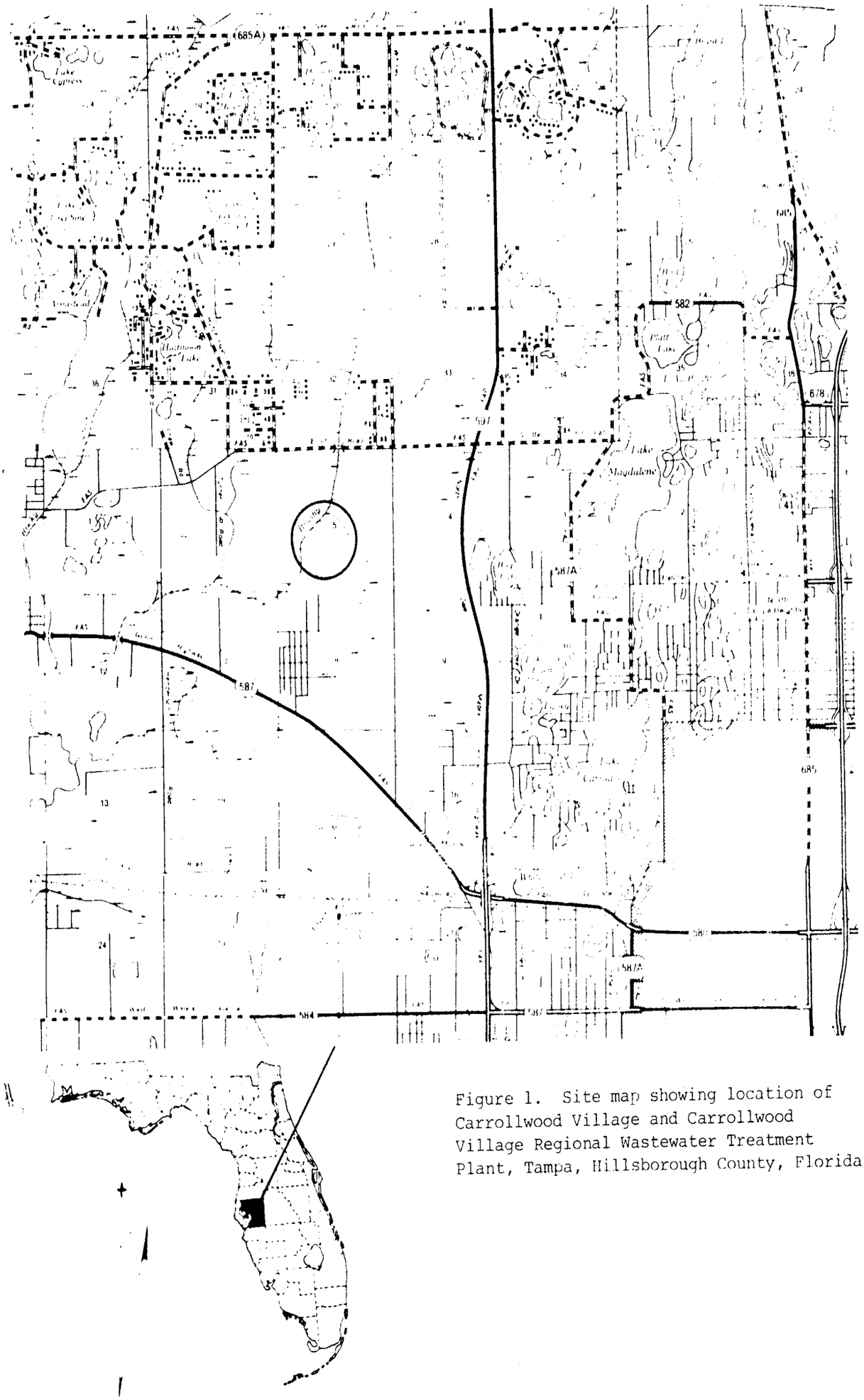


Figure 1. Site map showing location of Carrollwood Village and Carrollwood Village Regional Wastewater Treatment Plant, Tampa, Hillsborough County, Florida.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organisms selected for this study include species recommended by Peltier (1978) and the U.S. EPA (1978). Additionally, the Biological Section maintains cultures of several species of organisms indigenous to the State of Florida. Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

History and Maintenance of Bioassay Organisms

Daphnia pulex (water flea) - A breeding culture of Daphnia pulex was obtained from the EPA laboratory in Athens, Georgia in March, 1979. This culture has been maintained since its arrival, in 2½ gallon all glass aquaria filled with unchlorinated DER well water, using a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of $22^{\circ} \pm 1^{\circ}C$. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess daphnids. They are then refilled with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Simocephalus serrulatus (water flea) - An indigenous breeding population of S. serrulatus was collected from Lake Jackson, Leon County, Florida, in March, 1980, by personnel from DER's Biological Section. Positive identification of the organism was performed by Mr. Jeff Foran of the Department of Environmental Engineering Sciences, University of Florida in Gainesville. The cultures are maintained in 2½ gallon all glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. The organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of $22^{\circ} \pm 1^{\circ}C$. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess population. They are then refilled with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biological Section conducts monthly Standard Reference Toxicant Bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Three separate 48 hour static bioassays were conducted from 0855 hours 6 August to 0930 hours 8 August, 1980, on grab samples of effluent from the overflow structure into Brushy Creek, and from the clarifier before chlorination. The samples were collected by Mr. Bob Carpenter of the Hillsborough County Environmental Protection Commission at 0845-0900 hours 5 August 1980, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analysis were preserved with HNO_3 (2ml/liter). Samples for organic chemical analysis and for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee within 24 hours of collection.

Freshwater for dilution of the effluent sample was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study (Table 1) is that recommended by APHA (1976).

Two replicates, each with six concentrations and a control, were used to test with Daphnia pulex and with Simocephalus serrulatus. Flint glass jars of 250 ml capacity were used as test chambers, and 200 ml of the appropriate test concentration was placed in each chamber. Daphnia and Simocephalus were siphoned into a large culture dish through a series of nested stainless steel screens to isolate the proper size and age organisms (neonates). These neonates were then transferred individually by glass pipette and were loaded 10 per chamber, resulting in 20 per test concentration.

Dissolved oxygen, pH, and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. Total alkalinity, total hardness, and conductivity were recorded at the beginning of the test for the controls and the 100% effluent concentration. These values were recorded on the bioassay data forms and are presented in Tables 2, 3 and 4. Organisms were not fed during these tests.

Table 1. Concentration Series and Volumes Used for These Static Bioassays

Water flea (Daphnia pulex and Simocephalus serrulatus)

Concentration	Freshwater diluent	Effluent	Total Vol.
Control	200 ml	0 ml	200 ml
5.6%	188.8 ml	11.2 ml	200 ml
10.0%	180 ml	20 ml	200 ml
18.0%	164 ml	36 ml	200 ml
32.0%	136 ml	64 ml	200 ml
56.0%	88 ml	112 ml	200 ml
100.0%	0 ml	200 ml	200 ml

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Carrollwood Village Regional Wastewater Treatment Plant, Brushy Creek Outfall, Tampa, Hillsborough County, Florida, 6 to 8 August 1980.

Conc. or %	Test Container Number	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Conductivity (umhos)				
		0	24	48	0	24	48	0	24	48	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	A	10	10	10	8.8	8.9	8.3	7.5	8.9	7.8	114					117					335				
Control	B	10	10	10	8.8	8.8	8.3	7.5	7.8	7.8															
5.6%	A	10	10	10	8.9	8.8	8.4	7.5	8.0	7.9															
5.6%	B	10	10	9*	8.9	8.6	8.5	7.5	8.0	7.8															
10.0%	A	10	10	10	8.9	8.9	8.5	7.6	8.0	7.8															
10.0%	B	10	10	10	9.0	8.9	8.5	7.7	8.0	7.8															
18.0%	A	10	10	10	8.9	8.8	8.5	7.8	8.1	7.8															
18.0%	B	10	10	10	8.9	8.8	8.5	7.8	8.1	7.8															
32.0%	A	10	10	10	8.9	8.9	8.5	8.1	8.2	7.8															
32.0%	B	10	10	10	8.9	8.8	8.5	8.2	8.2	7.9															
56.0%	A	10	10	10	8.9	8.7	8.5	8.5	8.3	7.9															
56.0%	B	10	10	10	9.0	8.7	8.5	8.6	8.4	7.9															
100%	A	10	9	8	8.9	8.6	8.5	9.0	8.6	8.0															
100%	B	10	10	10	9.0	8.5	8.5	8.9	8.7	8.0	16/129 **					119					600				

Remarks:
1. Test temperature range 21.5° to 22.5°C.
2. Free chlorine in 100% effluent not detectable (Hach DPD) probably due to long retention time in a series of polishing ponds prior to discharge.
3. Algae present in effluent
   * 1 Daphnia stuck to glass just above waterline
   ** 16 = phenolphthalein alkalinity, 129 = total alkalinity

Table 3. Data recorded during 48-hour, *Simocephalus serrulatus* static acute toxicity bioassay of Carrollwood Village Regional Wastewater Treatment Plant, outfall to Brushy Creek, Tampa, Hillsborough County, Florida, 6 to 8 August 1980.

Conc. or %	Test Container Number	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Conductivity (µmhos)				
		0	24	48	0	24	48	0	24	48	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	A	10	10	6	8.8	8.8	8.3	7.5	8.0	7.8	114					117					335				
Control	B	10	10	10	8.9	8.8	8.4	7.5	8.0	7.8															
5.6%	A	10	10	10	8.9	8.8	8.4	7.5	8.0	7.8															
5.6%	B	10	10	10	8.9	8.8	8.4	7.6	8.0	7.8															
10.0%	A	10	10	10	8.9	8.8	8.4	7.7	8.1	7.8															
10.0%	B	10	9	9	8.9	8.9	8.4	7.7	8.0	7.8															
18.0%	A	10	10	10	8.9	8.8	8.4	7.8	8.1	7.8															
18.0%	B	10	10	10	8.9	8.8	8.4	7.8	8.1	7.8															
32.0%	A	10	10	9	8.9	8.8	8.4	8.2	8.2	7.8															
32.0%	B	10	10	10	8.8	8.8	8.4	8.2	8.2	7.8															
56.0%	A	10	10	10	8.9	8.7	8.4	8.5	8.4	7.9															
56.0%	B	10	10	10	9.0	8.8	8.4	8.6	8.4	7.9															
100%	A	10	10	10	9.0	8.5	8.4	8.9	8.7	8.0															
100%	B	10	10	10	9.0	8.5	8.4	9.0	8.7	8.0	16/129*					119					600				

Remarks:

1. Test temperature range : 21.5° to 22.5°C.
2. Free chlorine in 100% effluent concentration is not detectable (Hach DPD) due to long retention time in a series of polishing ponds.
3. Algae present in effluent.

* 16 = phenolphthalein alkalinity, 129= total alkalinity

Table 4. Data recorded during 48 hour, *Daphnia pulex*, static acute toxicity bioassay of Carrollwood Village Regional Wastewater Treatment Plant, effluent from clarifier before chlorination, Tampa, Hillsborough County, Florida, 6 to 8 August 1980.

Conc. or %	Test Container Number	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Conductivity (µmhos/cm)				
		0	24	48	0	24	48	0	24	48	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	A	10	10	10	8.9	8.3	8.3	7.6	8.1	7.7	114					117					335				
Control	B	10	10	10	8.3	8.5	8.5	7.6	8.0	7.7															
5.6%	A	10	9	9	8.6	8.4	8.5	7.5	8.0	7.7															
5.6%	B	10	10	10	8.7	8.6	8.5	7.5	8.0	7.7															
10.0%	A	10	10	10	8.5	8.6	8.4	7.5	8.0	7.7															
10.0%	B	10	10	10	8.6	8.5	8.4	7.5	8.0	7.7															
18.0%	A	10	10	10	8.1	8.4	8.4	7.4	8.0	7.6															
18.0%	B	10	10	10	8.2	8.2	8.4	7.4	8.0	7.7															
32.0%	A	10	10	10	7.5	8.2	8.1	7.3	8.0	7.6															
32.0%	B	10	10	9	7.6	8.3	8.2	7.3	8.0	7.6															
56.0%	A	10	10	7	6.4	8.1	8.2	7.2	7.9	7.6															
56.0%	B	10	9	8	6.5	8.0	8.1	7.2	7.9	7.6															
100%	A	10	9	4	4.2	7.8	7.9	7.2	7.9	7.6															
100%	B	10	7	5	4.4	7.8	7.9	7.1	7.9	7.7	178					181					850				

Remarks: 1. Test temperature range: 21.5° to 22.5°C.

RESULTS AND DISCUSSIONS

The results of assays performed on effluent from Brushy Creek Outfall of the Carrollwood Village Regional Wastewater Treatment Plant, using the water fleas Daphnia pulex and Simocephalus serrulatus, indicated that the effluent was not toxic to these organisms. However, tests performed on effluent collected at the clarifier before chlorination, using Daphnia pulex, indicated this effluent was toxic at 48 hours (48 hour LC_{50} = 91%, Table 5). The 96 hour LC_{50} would therefore be $\leq$ 91%.

Results of chemical analyses performed on the two effluent samples are presented in Table 6. The only unusual result noted was that heptachlor (0.03 µg/l) was found in the effluent sample from the clarifier. However, toxicity research on heptachlor produced a 48 hour LC_{50} of 42 µg/l for Daphnia pulex (Cope 1966, in U.S. EPA 1976). It is therefore difficult to speculate as to the cause of toxicity in the pre-chlorinated effluent sample since all chemical parameters tested for were in non-toxic concentrations. Effluent collected at the point of discharge was not toxic to the organisms tested which suggests that the toxic component(s) in the clarifier effluent was rendered non-toxic in the polishing ponds.

Table 5. LC_{50} Data for Bioassay of Northwest Hillsborough Regional Wastewater Treatment Plant, Tampa, Hillsborough County, Florida. 6-8 August, 1980.

Organism	Method of Data Analysis	24 hr LC_{50} and 95% confidence intervals		48 hour LC_{50} and 95% confidence intervals	
Daphnia pulex	Binomial		A		A
Assay #1	Moving Average		A		A
Brushy Creek Outfall					
No chlorine	Probit		A	< 100%	B
Simocephalus serrulatus	Binomial		A		A
Assay #2	Moving Average		A		A
Brushy Creek Outfall					
No chlorine	Probit		A		A
Daphnia pulex	Binomial		A	91% (56 and ∞)	B
Assay #3	Moving Average		A	91% (66 and ∞)	B
Pre-chlorinated sample	Probit	>100%	B	> 100%	B,C

A. Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, an LC_{50} value cannot be calculated using this method of statistical analysis.

B. Due to the distribution of mortality, the LC_{50} value calculated by this method of statistical analysis does not meet the criteria established by the Committee on Methods for Toxicity Tests with Aquatic Organisms.

C. Due to the distribution of mortality, results using the probit method are not statistically sound.

Table 6. Physical and chemical parameters of effluents from Carrollwood Village Regional Wastewater Treatment Plant, Tampa, Hillsborough County, Florida.

1. Brushy Creek Outfall:

Temperature:	30.5°C
Conductivity:	692 µmhos
pH:	9.5
Dissolved Oxygen:	8.7 mg/l
Total Residual Chlorine:	none detected (Hach DPD)
Cadmium:	< 1 µg/l
Copper:	< 5 µg/l
Zinc:	<25 µg/l
Total Chromium:	<10 µg/l
Mercury:	<0.2 µg/l
Phenols:	none detected
Chlorinated Hydrocarbons:	none detected
Pesticides:	

2. Clarifier Effluent Before Chlorination:

Temperature:	28.5°C
Conductivity:	944 µmhos
pH:	7.5
Dissolved Oxygen:	1.8 mg/l
Total Residual Chlorine:	none detected (Hach DPD)
Cadmium:	< 1 µg/l
Copper:	< 5 µg/l
Zinc:	<25 µg/l
Total Chromium:	<10 µg/l
Mercury:	<0.2 µg/l
Phenols:	<none detected
Chlorinated Hydrocarbons:	<none detected
Pesticides:	<0.03 µg/l Heptachlor

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