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Full Length Research Paper

Uptake and distribution of hexavalent chromium in tissues (gill, skin and muscle) of a freshwater fish, Tilapia, *Oreochromis aureus*

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Water pollution by heavy metals, especially chromium pollution from industrial sources can affect aquatic life, all ecosystems and human health directly or through food chain. This study aims to investigate the uptake of hexavalent chromium by a freshwater fish, (Tilapia, *Oreochromis aureus*). Short-term acute toxicity tests were performed over a period of 96 h providing the medium with various concentrations of potassium dichromate. Then the 96 h LC_{50} value was found to be 91.51 mg l^{-1} (Cr^{6+} as 32.35 mg l^{-1}). Five different concentrations of Cr^{6+} varying between 10, 15, 20, 25 and 30 mg l^{-1} were implemented for the uptake of this metal. The experiment was carried on for 28 days, meanwhile sampling fish weekly. With continued exposure, the accumulations were increased and fish progressively lost their ability to respond to this increase in exposure period. The chromium concentration in different organs was in the following order gill > skin > muscles tissues (least). The concentration of Cr in the gill range from 3.11 - 45.23 µg g^{-1} w.w, while the concentration accumulated in the muscle tissue of fish ranged from 0.86 to 12.34 µg g^{-1} w.w.

Key words: Heavy metal, Cr toxicity, fish, Tilapia (*Oreochromis aureus*), accumulation.

INTRODUCTION

Heavy metals are introduced into the environment by a wide spectrum of natural sources such as volcanic activeties, erosion and anthropogenic ones including industrial wastes as well as a leakage. Some of these metals including lead, nickel, cadmium, mercury are toxic to living organisms even at quite low concentrations, while others such as copper, iron, zinc and manganese are biologically essential and natural constituents of the aquatic ecosystems and become toxic only at very high concentrations (Cohen et al., 2001; Storelli et al., 2006; Karadede and Ünlü, 2007).

Chromium is considered as a heavy metal and pollutant as well as an essential micronutrient. Wastewater pollution by chromium originating from electroplating, dyeing, tannery, hard-alloy steel and stainless steel manufacture, has affected the life on earth. Chromium is also used as a catalyst and coating material (Idachaba et al., 2004). Welding, grinding and polishing of stainless steel are among principal ways of introducing chromium into the land environment while other ways of introducing chromium into air and water environments include the burning of fossil fuels and waste incineration (WHO, 1988). This pollution could affect all ecosystems and human health directly or through food chain (Yılmaz et al., 2009).

Chromium exists in different oxidation states which have distinct biological effects (Richard, 1991). Hexavalent chromium [(Cr VI)] is a well known carcinogen metal form for animals and human beings. Cr (VI) compounds readily penetrate into cell membranes via anion transport systems. It was clear from previous studies that Cr (VI) itself was highly active and carcinogen should it arrive as Cr (VI) inclusion to the target (Costa, 1997; Ding and Shi, 2002). Cr (VI) has the ability to lend electrons and be reduced to trivalent chromium [Cr (III)]. In contrast to Cr (VI), the trivalent form of Chromium is 500 to 1000 times less active against living cells because of its poor uptake (Alexander and Aaseth, 1995). Cohen et al. (2001) suggested that Cr (III) was much less active than Cr (VI) to a cell but when

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Cr (III) enters the cell, it could cause toxicity on a basis comparable to Cr (VI).

Fishes are often at the top of the aquatic food chain and may concentrate large amounts of certain metals from water (Mansour and Sidky, 2002). In addition to adsorption on tissue and membrane surface, fish may assimilate metals by ingestion of particulate material or food in water, or ion-exchange of dissolved metals through lipophilic membranes, e.g. the gills (Mendil et al., 2005). Metal distribution between different tissues depends on the mode of exposure, that is, dietary and/or aqueous exposure, and can serve as a pollution indicator (Alam et al., 2002). The objective of our study was to investigate the impact of different chromium (10, 15, 20, 25 and 30 mg l^{-1} Cr^{6+}) pollutions on the survival of juvenile *Oreochromis auras* (Tilapia) and to compare the chromium concentrations in fish tissue (muscle, skin and gill).

MATERIALS AND METHODS

Fish

O. aureus (Tilapia), a tropical freshwater and important culture fish, is a specie commonly found in brackish water in estuaries all over the world and responds promptly to environmental alterations (Vijayan et al., 1996; Almeida et al., 2002; Turan, 2006). Juvenile specimens of Tilapia were captured from ponds at Çukurova University Aquaculture Research Centre and transferred to the laboratory where the experiments were conducted. Holding and acclimatization took place in tanks containing fresh water at temperature of 20 ± 2°C and oxygen content 80%. The samples were placed in aquarium for one week to allow for adaptation of the fish to the new conditions. Tap water used for the experiment had a pH value of 7.6 ± 0.3 and total hardness of 135 ± 5.8 mg $CaCO_3$ l^{-1}. The aquariums were aerated with air stones for proper oxygen saturation (8.7 mg O_2 l^{-1}). Fish were fed once a day with artificial feed meal. Acclimatized fish were moved at random into test aquaria (40 x 40 x 80 cm) as well as into control aquaria, in four aquaria for each concentration and control, containing twelve fishes.

Chemicals

All reagents were analytical grade. Required concentrations of potassium dichromate (Merck) were prepared by adding aliquots of 1% stock solution in double distilled water. A Cr (VI) stock solution was delivered to twenty test aquariums via automatic pipettes. The toxicant solution in the aquarium was replaced with fresh solution of the same concentration every 24 h. Renewal bioassays were conducted using five concentrations of potassium dichromate (Cr^{6+} as 10, 15, 20, 25 and 30 mg l^{-1}). Controls without toxicant were also run simultaneously.

Experimental exposure

Treatment water was monitored every day for dissolved oxygen, pH and Cr concentrations. Exposure period was 28 days, during which samples were taken on day 7, 14, 21 and 28.

Experimental units were checked daily for mortality and behavioral changes and any dead fish was immediately removed from the aquariums. For measurement purpose, 3 fishes were taken from each replicate aquarium and their lengths were measured (cm) and weighed (g) before dissection with cleansed tools.

Water and fish tissue analysis

Samples of 100 ml of water from each treatment were filtered through a 0.45 µm micropore membrane filter and kept at -20°C until analysis process. Each filtered sample was transferred to a pre-cleaned polyethylene bottle, acidified to 1% HNO_3 and analyzed with Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, Varian model- Liberty Series II). The absorption wavelength and instrument detection limit were 283.553 nm and 0.007 µg l^{-1}. For the analyses, the gill, a part of skin and approximately 5 g of the epaxial muscle on the dorsal surface of each fish were dissected, washed with distilled water, weighed, packed in polyethylene bags and stored at -20°C prior to analysis. The digestion of the fish tissues was in accordance with methods described by Yilmaz (2003) with concentrated HNO_3/HCl (1:3 v/v). Metal concentrations were determined with ICP-AES. Standard reference materials were as follows: Multi-4 Merck for fish, SRM-143d for water acc. to National Institute of Standards and Technology. The results indicated good agreement between the certified and analytical values with recovery rates of Cr between 92 and 103% for fish, 94 and 102% for water. The concentrations were expressed as micrograms per gram wet weight (µg g^{-1} wet wt.) of tissue in organisms.

Statistical evaluation

For the survival tests, Statistical Analysis of data was carried out with SPSS statistical package program. A value of $p < 0.05$ was considered to be significant. For the accumulation tests, the experiments were repeated four times and only the arithmetic mean of the four experiments at each concentration was taken to express the results.

RESULTS

Test organisms (n = 300) were juvenile specimens of Tilapia of mean lengths 10.2 ± 1.3 cm and weights 18.39 ± 2.09 g. There was no statistical difference between the study groups and control group regarding the size of the fish ($p > 0.05$).

Physico-chemical parameters of fresh water quality including pH value, temperature and the amount of dissolved oxygen of the test aquariums were determined as 7.06 ± 1.24, 21.8 ± 2.02°C and 8.18 ± 1.46 mg l^{-1} respectively during the experiment. Higher metal concentrations caused a significant decrease in pH values, however, in all cases; pH values did not reach the acidic range, which could affect the organisms' survival.

The concentrations of Cr measured in the aquariums throughout the experiment were ± 10% of the nominal concentrations (Table 1). Therefore, nominal concentrations from now on will take this value as the basis. The general agreement of Cr^{6+} with nominal concentrations confirmed that virtually all of the Cr remained in the Cr^{6+} form throughout the experiment (Farag et al., 2006).

Table 1 summarizes the survival of the fish during a 4-week-exposure period for juvenile specimens. Survival of control fish was nearly 100% throughout the experiment. Chromium concentrations (25 and 30 mg l^{-1} Cr^{6+}) reduced

Table 1. Mean of measured chromium concentration in water (Cr^{6+} µg l^{-1}), standard deviation (SD) and mean percent survival of juvenile Tilapia (*O. aureus*) exposed to Cr^{6+} as $K_2Cr_2O_7$ during 28 days.

Theorical chromium concentration (Cr^{6+} µg l^{-1}) in water	Mean of measured chromium concentration in water (Cr^{6+} µg l^{-1}) (SD)	Initial number of Tilapia per aquarium	Percent survival in exposure days (d)			
			7th d	14th d	21th d	28th d
Control (0)	1 (0.1)	12	100[a]	100[a]	95.8[a]	95.8[a]
10	9.7 (0.7)	12	95.8[a]	97.2[a]	95.7[a]	90[a]
15	13.9 (0.5)	12	95.8[a]	94.1[a]	85[a]	80[b]
20	19.8 (0.8)	12	93.9[a]	93.9[a]	89.5[a]	80[b]
25	24.4 (0.9)	12	89.6[a]	87.1[a]	80[b]	62.5[b]
30	28.9 (1.2)	12	87.5[a]	76.7[b]	71.4[b]	54.5[c]

Different superscript letters (a, b and c) designates difference at $p < 0.05$ within a sample day.

fish survival beginning from the first week of experiment, where high mortality rate of Cr-exposed fish occurred within 14 to 21 days. Survival rate decreased to 54.5% in fish exposed to 30 mgl^{-1} Cr^{6+} dose from days 21 to 28. The behavioural changes of the control group and Tilapia exposed to various doses of K_2CrO_4 were compared with each other during the experiments. The control group displayed normal behavior during the test period. The lowest concentrations (10 and 15 mg l^{-1} Cr^{6+}) had similar behavior to that of the control group. From the dose 20 mg l^{-1} Cr^{6+}, the fish started to show behavioral disorders such as loss of equilibrium, sudden startling and respiratory difficulties. 25 mg l^{-1} Cr^{6+} and at the highest concentration (30 mg l^{-1} Cr^{6+}) onwards, there was shivering, rather high respiratory disorder and swimming in capsized position.

The accumulations of chromium (µg/g w. wt) in the gill, skin and muscle tissues of Tilapia during the exposure period are shown in Figures 1a - e. The initial Cr^{6+} concentrations were: 1a, 10 mg l^{-1} ; 1b, 15 mgl^{-1} ; 1c, 20 mgl^{-1}; 1d, 25 mg l^{-1}; 1e, 30 mg l^{-1}. The chromium concentrations in the tissues of control fish were below the detection limit of the instrument (< 0.007µg l^{-1} Cr^{6+}) throughout the experiments. The concentrations (µg g^{-1} wet weight) of Cr in the organs (gill, skin and muscle) of fish increased when they were exposed to Cr in the water (Figure 1a - e). Chromium accumulation in the muscle tissue of fish ranged from 0.86 to 12.34 µg g^{-1} w.w., while concentrations in the gill were within the range of 3.11 - 45.23 µg g^{-1} w.w. (Figure 1a - e).

As can be seen in Figure 1a, maximum level of Cr^{6+} was 1.30 µg g^{-1} w.w on fish muscle having lived 28 days at 10 µg l^{-1} Cr^{6+} initial concentrations. On the other hand, higher concentration of Cr^{6+} (12.34 µg g^{-1} w.w.) was observed on fish muscle after 28 days at 30 µg l^{-1} Cr^{6+} (Figure 1e). Maximum levels of Cr^{6+} concentrations on the muscle tissues at 15 (after 28 days) in Figure 1b, 20 (after 28 days) in Figure 1c and 25 (after 28 days) µg l^{-1} Cr^{6+} treatments in Figure 1d were 2.43, 9.03 and 10.81 µg g^{-1} w.w., respectively. The concentrations of Cr in gills were almost twice, three and four times as high as in the muscle at 20, 25 and 30 µg l^{-1} Cr^{6+} mediums, respectively. The Cr accumulation on the skin of Tilapia for all experimental concentrations was nearly twice as much as in the muscle of samples at the end of experiment.

DISCUSSION

It is widely known that metal toxicity is more accurately measured in fresh water than in sea water, because metal appear to a great extent as complex compounds in sea water and this reduces the toxicity of metal ions. The reaction and survival of aquatic animals depend not only the biological state of the animals and physico-chemical characteristics of water (such as pH, temperature, hardness and the amount of dissolved oxygen) but also on the kind, toxicity, type and the duration of exposure to the toxicant (Martin et al., 1981; Mays, 1996; Vutukuru, 2005). In the present study, the mortality increased with an increase in concentration and also the duration of the exposure. This may be rather significant, because smaller fish being generally more active than larger ones, metal uptake and elimination of metal could also occur in higher rates in these smaller ones (Heath, 1987; Larsson et al., 1985; Canli and Furness, 1993).

A comparison of toxicity values for 10 µg l^{-1} Cr^{6+} to 30 µg l^{-1} Cr^{6+} experiments demonstrated a decline in survival rate following longer exposure periods. Even thought the magnitude of this decline varied between the different chromium concentrations, lower (10 to 20 µg l^{-1} $Cr^{6+)}$ mediums were about 10 to 20% mortality while higher (25 and 30 µg l^{-1} $Cr^{6+)}$ mediums were 38 to 45% mortality at the end of experiments. Nearly no mortality was recorded in the experiment controls.

The World Health Organization (WHO), U.S. EPA, as well as nearly every state agency (Turkish Environmental Guidelines, Class I-II, 1998; TSE 266) has set the drinking water standard for Cr (VI) at 50 or 100 ppb. These parameters (Cr (VI)) are not a small amount of Cr (VI) since it would be equated to 1 to 2 µM Cr (VI) (Costa, 2003). The Cr concentrations [10 to 30 µg l^{-1} (^{ppb}) Cr (VI)]

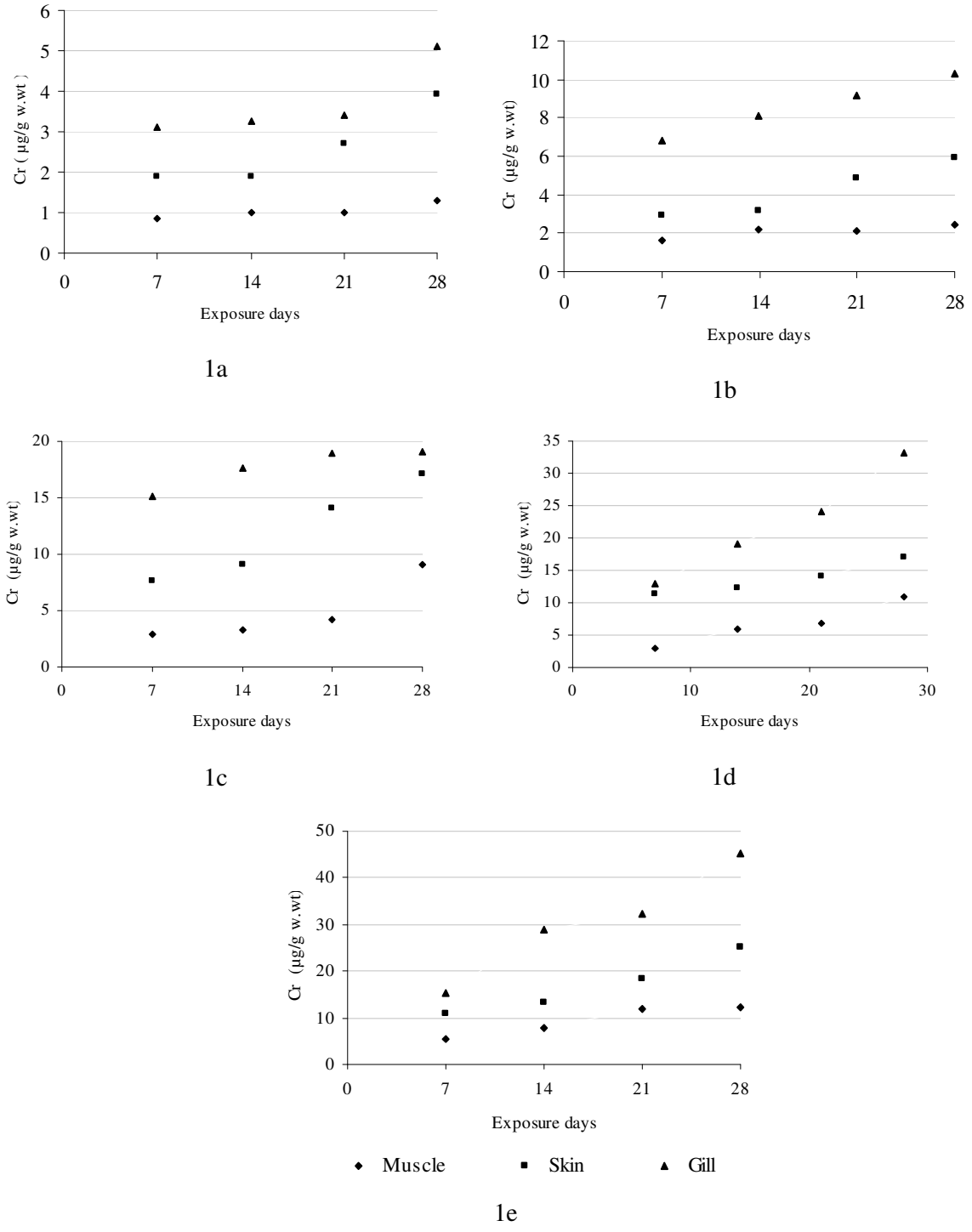

Figure 1. Time-dependent bioconcentration levels of chromium µg per gram wet weight (µg/g w. wt.) in muscle (♦), skin (■) and gill (▲) tissues of Tilapia. The initial Cr^{6+} concentrations: 10 mg l^{-1} (a), 15 mg l^{-1} (b), 20 mg l^{-1} (c), 25 mg l^{-1} (d) and 30 mg l^{-1} (e).

used in the present study has been proved not to be sub-lethal for juvenile Tilapia and have been considered as representatives of environmental exposure. However, high Cr concentrations indicate that effects on survival were related to exposure concentrations and duration. At physiological pH, hexavalent Cr exists as an oxyanion and in this form, it resembles oxyanions such as sulfates and phosphates, which are used extensively in various

biochemical processes. The individual cells of the body, which have active transport systems for these nutrients, take up sulfates and phosphates (Costa, 2003). In this way Cr (VI) can enter the body. Vutukuru (2005) showed that chromium induced alterations at the biochemical level, more pronounced changes occurring at the end of 96 h and thus it was time-dependent. Furthermore, metal induced alterations in the protein content may probably affect the enzyme mediated bio defence mechanisms of the fish.

In the present investigation, the tendency of the body surface to acquire a dark color appears to be the first symptom of toxication in these fish just like in the previous study for cadmium (Hilmy et al., 1985). At the end of the experiment, fish showed externally abdominal distention and hemorhagic (reddening) anal-uregenital pours. Erosion and fusion were also detected in lamellar epithelium of gills.

The effects of hexavalent chromium on aquatic living things have been evaluated by several studies. Some data exist on the effects of Cr (VI) on salmon (Buhl and Hamilton, 1991; Farag et al., 2006). Other researcher studied heavy metal accumulation in tissues of Tilapia, a freshwater fish (Ay et al., 1999; Wepener et al., 2001; Aleya et al., 2005; Zirong and Shijun, 2007). However, no study has yet assessed chromium exposure and accumulation on juvenile Tilapia. Previous studies did not investigate accumulation of this metal on skin and gill as a result of Cr (VI) exposure (Arillo and Melodia, 1988; Buhl and Hamilton, 1991; Outridge and Scheuhammer, 1993; Farag et al., 2006).

It was noted that Cr accumulation in the tissues showed the following sequence: gills > skin > muscle tissues (least). As in the present study, Storelli et al. (2006) found that the lowest concentration of Cr was detected in the muscle tissue and skin and the gills showed similar levels ($p > 0.05$). Some researchers found metal accumulation in the tissues of rainbow trout *Salmo gairdneri* in the following organs: lead in the bone, spleen and kidney; chromium in the spleen, muscle and gills; copper in the kidney; zinc in the gills (Camusso et al., 1995). Yılmaz (2003) indicated that concentrations of heavy metals on wild fish were higher in all of the skin samples than in the muscles.

The reason for high metal concentrations in the skin could be due to the metal complexion with the mucus that is impossible to remove completely from the tissue before analysis. In general, higher metal concentrations in gills reflect metal concentrations of the water where the fish live. Gills are vital respiratory and osmoregulatory organs and cellular damage induced by the metal might impair the respiratory function of the fish by reducing the respiratory surface area (Vutukuru, 2005). Gills are regarded as an important part for direct metal uptake from the water whereas the body surface is generally assumed to play a minor role (Pourang, 1995). It was demonstrated by Wepener et al., (2001) that gill tissue in banded tilapia was the initial site of accumulation of waterborne metals. The tendency of the metal to bind to the external gill surface was via ionic bonds, and to gill cytosolic compounds is via covalent bonds. It was also observed that the heavy metals in muscle tissue were at low levels compared with other organs (Sağlamtimur et al., 2003; Karadede and Ünlü, 2007). The results also show that chromium is more accumulated in the samples of gill than in the skin or muscles.

For the Cr metal, the European legislation has not established maximum level and therefore, an evaluation of the chemical quality of this fish is only possible utilizing dietary standards fixed in other countries. For example, the Western Australian Food and Drug Regulation List limit the level for Cr 5.5 $\mu g\ g^{-1}$ (Usero et al., 2003). As it was observed that fish could live even at higher levels than those mentioned in EPA standards, it is safe to say that fish can cope with a higher accumulation rate of heavy metals in their bodies, which itself is not safe for human consumption. Therefore, it would be advisable to take into consideration the duration of contact with toxic substances and the fish species in addition to concentration rate before establishing a basis for permissible levels in environmental studies.

Conclusion

The results in the current study showed that the exposure of Tilapia to waterborne Cr (VI) caused significant accumulations in their organs and the accumulations were associated with the exposure period, concentrations of chromium and different tissues. Even studied levels of Cr (VI) were below drinking water standard (50 or 100 ppb) that the accumulations on gill, skin and also muscles were high. With longer exposure, the accumulations were increased and fish progressively lose their ability to respond with the increase in exposure period. These malfunctions are particularly important because they are associated with changes in survival, which can be related to effects at the accumulation levels. Future research should focus on the effect of chromium toxicity on bio defence mechanisms of Tilapia at sub-lethal levels.

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