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Potential Nephrotoxic Effect of UV-328 and Its Possible Salvage by Dimethoxy Curcumin in Zebrafish

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The occurrence of Benzotriazole UV Stabilizer-328 (UV-328) in different environmental and natural systems is of fast regular concern these days. In this current paper, we assessed the renotoxicity of UV-328 in zebrafish kidney tissues to know the chore of oxidative devilry in kidney and to recuperate the renotoxicity utilizing Dimethoxy curcumin (DiMC) supplementation. Grown-up zebrafish were exposed 55µg/L of UV-328 and DiMC supplemented through diet at 50mg/kg BW. Close to the completion of 28 days, renal tissues were examined for the responses of oxidative pressure, antioxidant status and histopathological changes. The results demonstrated that antioxidant enzymes such as, Glutathione (GSH) levels and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) were all reduced in zebra fish kidneys treated with UV-328. Renal malondialdehyde (MDA) levels was

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brought prominently up in the UV-328 treated fish. All of the altered variables significantly returned to near-normal levels in the DiMC-supplemented group. Histopathological lesions, viz., hypertrophied glomerulus, cytoplasmic and nuclear degeneration, cytoplasmic vacuolization, degeneration of renal tubules were seen in UV-328 treated kidney of zebrafish which were actually reinforced in the DiMC treated fish. From our outcomes, it has been proposed that even at the low-level convergence of UV-328 exposure is malicious and to provoke oxidative insult, cell reinforcement exhaustion and kidney neurotic devilry in zebrafish and remediation with DiMC has been ended up being the better choice to conquer the harmful oppression prompted by UV-328.

Keywords: UV-328; oxidative stress; antioxidants; histopathology; zebra fish.

1. INTRODUCTION

UV-328 is a high-volume added substance regularly utilized as a Ultra violate stabilizer in plastic items, mirror coating, skin care products and rubber coatings. It has been tracked down in the climate and biota, a long way from its creation and use, remembering for distant areas of arctic and the Pacific seas. UV-328 has been viewed as shipped with, and may accordingly be let out of plastic trash. They may then be taken up by sea birds with ensuing collection in their tissue, and microplastics. It is additionally the first non-halogenated synthetic compound thought about by the persistent organic pollutants (POP's). Pair to high creation volume and far and wide applications, it has become pervasively spread in many ecosystems through manufacturing plants, plastic and rubber factories and wastewater treatment plants [1,2].

Because of its photostability and high hydrophobic nature, it has been generally utilized in private cleanliness items, building materials, auto parts, athletic gears, films, stains and plane defogging liquids to forestall yellowing and light-prompted debasement [3,4]. It is profoundly insistent, bio accumulative and modestly harmful natural material. On the other hand, it has been reported to impose negative effects on fish's antioxidant defense system. Besides, Denghel et al. [4] have explored the oxidative insult of UV-328 in an in vitro model including human kidney microsomes. Albeit a few examinations have stressed the harmful impacts of UV-328 on life forms, the constant impacts of UV-328 on zebrafish (*Danio rerio*) have not been exhaustively investigated. Therefore, a multi-biomarker approach was used in this study to address the negative biochemical responses of fish that are associated with UV-328 contamination in order to determine whether and how UV-328 affects the renal system of zebrafish.

It is deep rooted that openness to a far reaching of ecological impurities like POP's can evokes the development of reactive oxygen species (ROS) and upsurge the degree of cell oxidative pressure in aquatic fauna. Zebrafish own a multifaceted safeguard framework to protect themselves from oxidative affront. Expressly, fish cell reinforcement defense, for example, SOD, CAT, GPx and GST are referred to function as the essential line of enzymatic safeguard against oxidative burden. These enzymes are sensitive to eco-poisons and are repetitively used as a device in natural hazards evaluation [5]. Also, a staggering creation of ROS by xenobiotics can react with cell macromolecules, especially through lipid peroxidation, protein carbonylation and forming nucleic acid adducts. Malondialdehyde (MDA), a final product of lipid peroxidation, can be utilized to examine the level of membrane harm in cells. Furthermore, the over creation of ROS upon persistent openness to UV filters are likewise responsible for the cell reinforcement depletion and disintegration of enzymatic antioxidant safeguard prompting oxidative insult in the tissues of the exposed organisms [6].

Zebrafish is a discerning model organism with concrete history of purpose for hazardous testing and assessment in ecotoxicological studies. The zebrafish can be utilized as a potential model fish in ecotoxicology examinations and its genome has been sequenced and has a general closeness to the human genome is around 80%.in qualities connected with sicknesses, making zebrafish, a considerate biomedical model organism [7]. Dimethoxycurcumin (DiMC) is a structural equivalent of curcumin in which methoxy groups replaced with the phenolic-OH groups. It has a symmetric design and is synthetically steadier than curcumin with expanded cell reinforcement, apoptotic viability as well as less harmfulness in ordinary cells and expanded metabolic firmness (bioavailability). Plasma levels for dimethoxycurcumin were

generally reach higher (3-times) in comparison with curcumin at same infused portion of 5 mg/kg BW in mice. In examination with curcumin, digestion of DiMC is less comprehensive and subsequently it is conceivable that DiMC displays an expanded antioxidant potential over curcumin [8].

Our past reports unveiled that constant UV stabilizers exposure prompted assorted biochemical and multiple organ deficits in the zebra fish model [9,6]. Hence, we predicted that persistent exposure to UV-328 at sub lethal level to cause oxidative renotoxicity in grown-up zebrafish and its recuperation through the supplementation of DiMC. To test this hypothesis, we treated zebrafish to 55µg/L of UV-328 and 50mg/kg, BW of DiMC through diet for four weeks and to assess the renal harmfulness of UV-328 and conceivable recuperation with DiMC supplementation. In particular, markers related with oxidative insult and antioxidant pathway enzymes were assessed to uncover plausible hidden detrimental toxic mechanism of UV-328 and the defensive impact of DiMC in UV-328 actuated renal shortages in zebrafish.

2. MATERIALS AND METHODS

2.1 Chemicals

Benzotriazole UV stabilizer - 328 (BUV-328; 98% purity) was obtained from Sigma-Aldrich, USA. Dimethoxy curcumin (DiMC) was supplied by Biosynth Ltd, UK as a gift sample. Dimethyl sulphoxide (DMSO) supplied by Sigma-Aldrich (USA) was utilized to prepare the stock solutions of BUV-328. The other chemicals employed in the present investigation were of analytical grade and used without any further purification.

2.2 Experimental Set-up

Adult wild-type (AB strain) Zebrafish (*Danio rerio*) with a body length of 2.46 ± 0.04 cm and a mean body weight of 0.28± 0.04 g were obtained from Mass biotech zebrafish facility, Chennai. The fishes were adjusted to the research facility conditions for a week time in glass aquarium going before to the trials as per the rules of the Association for Monetary Co-activity and Improvement (OECD (Association for Financial Co-activity and Advancement), 1996). The fishes were raised in re-circling circulated air through freshwater kept up with at 26 ± 1 °C, with a photo-period time of 12:12 h (light/dark) routine. During the acclimatization time frame, they were taken care of with fish food at not obligatory and water reestablishment was done one time each day. After acclimation, fish (450 numbers) were arbitrarily isolated into three trial gatherings, for example, water control group, UV-328 treated group at centralization of 55µg/L. Furthermore, UV-328 with DiMC treated group were treated with a similar grouping of UV-328 alongside DiMC 50mg/kg BW through the feed regimen. Each group was kept up with in three duplicates and each recreate contains 50 fish in 25 L test arrangement. Stock arrangements of UV-328 were arranged newly in DMSO. In order to maintain the aquarium's water quality and the appropriate concentrations of UV-328 and DiMC, the test solutions were changed every 24 hours. Prior to the experiment, they were starved for 24 hours. On day 28, fish were haphazardly chosen from openness and control and treated tanks (n = 15/repeat) and kidney samples were gathered and utilized promptly for biochemical examination. One more arrangement of kidney tissues was fixed in 10% formalin for histological perception.

2.3 Biochemical Analysis

The kidney tissues were flushed, homogenized with 50 mM super cold potassium phosphate cradle (pH 7.0), centrifuged for 10 min (10,000 rpm) and the unmistakable supernatant was gathered to gauge the protein content, chemical exercises (Grass, CAT, GPx and GST) GSH and MDA level. Each examine was acted in sets of three. GSH level was examined by the strategy for Moron et al. [10] and expressed at a density of g/mg protein. SOD movement was assessed (Marklund and Marklund) [11] by estimating the hindrance of pyrogallol autooxidation at 420 nm, and the catalyst action was communicated as Units/mg protein. CAT movement was assessed by estimating the absorbance of hydrogen peroxide at 590 nm and communicated as µmol H_2O_2 consumed/min/mg protein [12]. GST not set in stone [13] after the complexation of glutathione (GSH) with 1-chloro-2, 4-dinitrobenzene CDNB at 340 nm, and the outcome was given in µmol of CDNB form shaped/min/mg protein. GPx movement was assessed [14] after the oxidation of glutathione (GSH) within the sight of H2O2 at 412 nm and the information was communicated as µg GSH shaped/min/mg protein. MDA content was assessed by Devasagayam et al. [15] at 532 nm, which depends on 2-thiobarbituric corrosive (4,6-dihydroxypyrimidine-2-thiol; TBA) reactivity, and the outcome was communicated as nmol/mg protein. Lowry et al.'s method was used to

determine the protein concentration [16] with serum albumin as the reference standard.

2.4 Histopathological Investigation

Kidney tissues were at first fixed in 10% unbiased cradled formalin. The attired tissues were dried out in a progression of absolute ethanol, implanted in paraffin wax, segmented at 5µm thickness, and stained with hematoxylin and eosin (H&E) for histopathological examination [17-19]. The segments were inspected and shot utilizing a light magnifying lens.

2.5 Statistical Analysis

Statistical investigation was completed by utilizing GraphPad Crystal 5.0 programming software (GraphPad Programming Inc., San Diego, CA). The outcomes acquired from each exploratory groups were exposed to one-way investigation of difference (ANOVA), trailed by Dunnett's post-hoc correlation. Upsides of $P < 0.05$ were viewed as genuinely significant between the thought about groups.

3. RESULTS

No mortality was seen during the acclimatization and openness periods, and there was no tremendous distinction between the blank (water) and dissolvable control (DMSO) for any of the biomarkers during the exposure. Thus, the water control group was kept up with as the reference group.

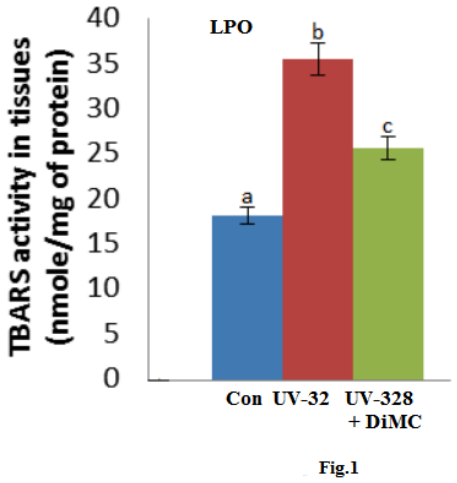
3.1 Impacts of UV-328 on Cell Reinforcement Enzymatic Exercises and Lipid Peroxidation

The activity of SOD, CAT, GPx and GST and the degree of GSH in the kidney of zebrafish presented to UV-328 are portrayed in the Table 1 and in the Figs. 1&2 separately. It is clear that when contrasted with the control, the activities of antioxidant enzymes and the GSH level were altogether diminished when exposed to UV-328 for 28 days. These adjusted variables were essentially recovered in DiMC treated group means the expected defensive role of DiMC against UV-328 prompted renal oxidative stress and keeps up with attuned antioxidant status. The fact that the MDA level was significantly higher in UV-328-treated renal samples and significantly lower in DiMC-supplemented renal tissue (Fig. 1) demonstrates DiMC's antilipoperoxidative properties.

Table 1. Effect of DiMC on UV-328 induced changes in the renal enzymatic antioxidants of Zebra fish

Experimental Groups	Kidney
SOD (Units/mg protein)	
Control	31.02 ± 1.43^{a}
UV-328	24.72 ± 1.98^{b}
UV-328+ DiMC	28.07 ± 1.75^{c}
CAT (µmol of H_2O_2 consumed /min/mg protein)	
Control	21.67 ± 0.65^{a}
UV-328	16.49 ± 1.27^{b}
UV-328+ DiMC	19.53 ± 0.97^{c}
GPx (µmol of GSH oxidized/min/mg protein)	
Control	11.54 ± 0.34^{a}
UV-328	$6.\ 31 \pm 0.61^{b}$
UV-328+ DiMC	9.20 ± 0.57^{c}
GST (µmol of CDNB conjugate formed/min/mg protein)	
Control	0.057 ± 0.007^{a}
UV-328	0.031 ± 0.002^{b}
UV-328+ DiMC	0.048 ± 0.005^{c}

Values are expressed as mean ± SE. The letters (a, b and c) indicate significant differences from the control and experimental groups determined by one way analysis of variance followed by Dunnett's post-hoc comparison, $p < 0.05$ (DMRT)



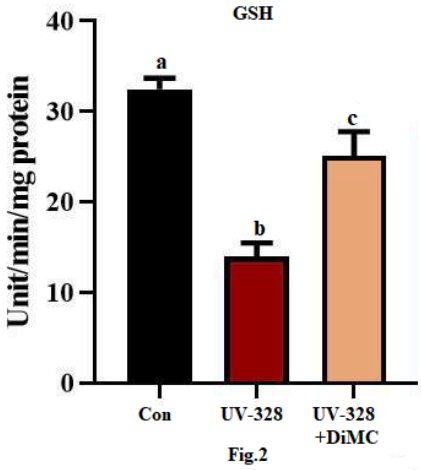


Fig. 1. Effect of UV-328 and DiMC on the LPO Fig. 2. GSH content in the kidney of zebra fish

Values are expressed as mean ± SE. The letters (a, b and c) indicate significant differences from the control and experimental groups determined by one way analysis of variance followed by Dunnett's post-hoc comparison, $p < 0.05$ (DMRT)

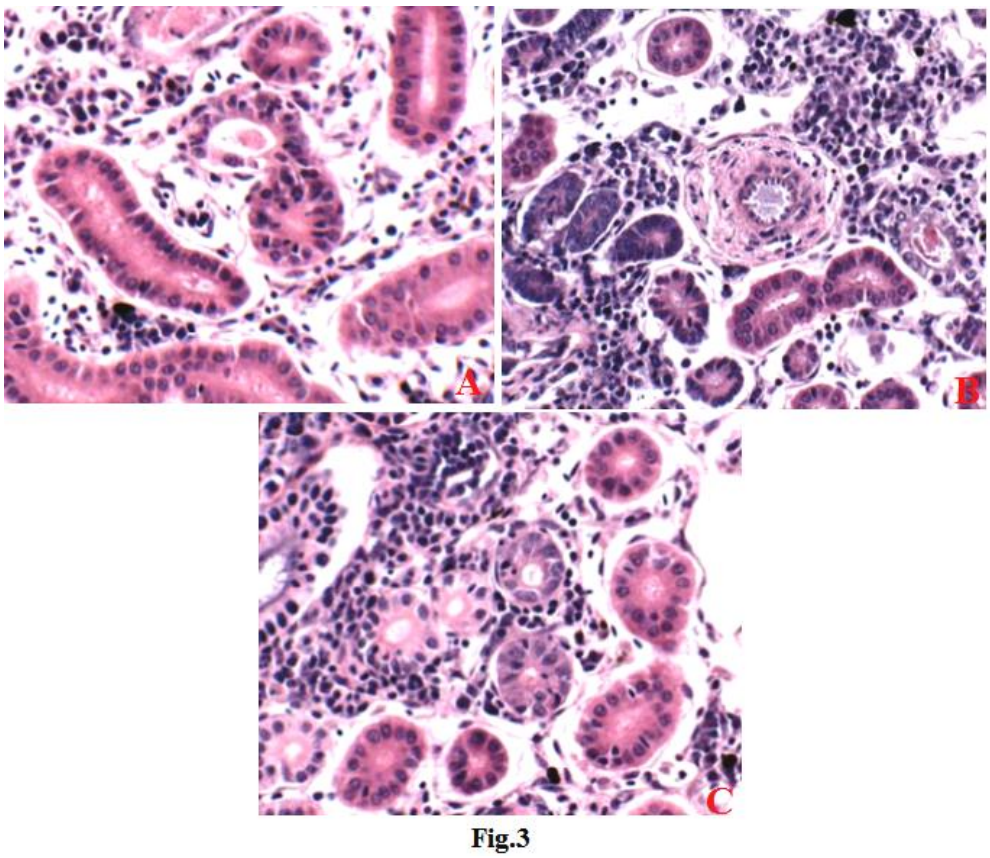


Fig. 3. Light micrographs of sections through kidney of zebrafish showing histological structure of the control and experimental groups

Samples were stained with hematoxylin and eosin and photomicrographs were taken using 400× magnification. (A). Control group showing the rigid morphological features of renal tissue with glomerulus and renal tubules. (B). UV-328 exposed renal tissue showing the altered pathological features like necrosis, desquamation, inflammation of renal structure. (C). UV-328 + DiMC treated renal tissue of Zebra fish showing almost normal features of the glomerular and tubular organization

3.2 Histopathological Changes

To explain the toxicity of UV-328 in a superior manner, the histological photomicrographs of kidney segments zebrafish have been displayed in Fig. 3. The renal morphology of the control group seemed, by all accounts, to be typical with practically no obsessive adjustments. It showed regular glomerulus, lymphoid tissues, distal and proximal tubules, and intercapsular spacing (Fig. 3A). However, some histopathological changes were observed in the UV-328 exposed group, including dilated glomerular capillaries, tubular obstruction and swelling, highly disorganized tissue architecture, an increase in Bowman's capsule space, and degenerated renal tubules (Fig. 3B). The fish in the DiMC-supplemented group displayed renal architecture that was nearly close to normal and lacked any pathological abnormalities, indicating the protective effect DiMC's (Fig. 3C).

4. DISCUSSION

Natural UV filters have a legitimate penchant to be delivered into oceanic climate and collected by pecking order organic entities, possibly jeopardizing fish species ripeness and capacity to recreate. The consequences of this current examination proposed that drawn out openness to UV-328 may make oxidative harm to fish kidneys and it very well may be conceivably be enhanced with the mediation of DiMC, a possible cell reinforcement.

4.1 Antioxidative Status

A methodology that is habitually used to survey the condition of oxidative stress is the estimation of the effects of xenobiotics on life forms through adjustments in a cell reinforcement catalysts and lipid peroxidation in tissues. SOD catalyzed the change of $O_2^{-\bullet}$ into H_2O and H_2O_2, and CAT/GPx are liable for the dismutation of H_2O_2 into water. They were going about as the main line of protection against oxidative stress in tissues. The phase II detoxification metabolic enzyme GST is able to bind the electrophilic groups of xenobiotics to the sulfydryl groups of GSH, making the toxic metabolites more hydrophobic. Lipid peroxidation can be restrained by GST and GPx activity [9]. When zebrafish were exposed to the organic filter UV-328, the activity of renal antioxidant enzymes drastically decreased in comparison to the control group. The explanation may be because of a superfluous quantity of ROS release, beyond what the antioxidant catalysts could dismutate. Comparable outcomes were additionally proclaimed by Velanganni et al. [6] in which antioxidant enzymes viz., SOD, CAT and GST levels in the kidney of zebrafish diminished which demonstrating that they took part in the detoxification of UV-328 toxic metabolites. These outcomes demonstrated that GST was engaged with the bio-transformation of UV-328, and the complex of GST and UV-328 was delivered to detoxify the toxics in zebrafish kidney. GSH detoxify not just by acting about as a substrate for GPx and GST, yet additionally by straightforwardly restricting the release of ROS and electrophilic compounds.

Previous assessments have obviously exhibited that exposure to natural contaminants brought about the increment or decline of GSH levels in test organisms relying upon the species, exposure concentration and duration [6,9,20]. The increased uptake of amino acid substrates and the activities of biosynthetic enzymes to protect organisms from oxidative damage likely contributed to the significant changes in GSH levels found in the UV-328 treated group in this study. The utilization of GSH because of the direct searching of ROS or as a co-factor for GST/GPx activity may essentially be responsible for the decline in the levels of GSH in the UV-328 treated group. Additionally, the response of GSH during xenobiotic exposure could have been impacted by its detoxification and sparing effects. This proposes that GSH might be more delicate to low dosages and reasonable for assessing the antioxidant status upon exposure to UV-328.

4.2 Lipid Peroxidation

Xenobiotics prompt zebrafish to create an enormous number of oxygen free radicals and will join with unsaturated fats in bio membrane and cause lipid peroxidation. When aquatic species are exposed to pollutants, MDA is frequently used as an impelling biomarker to evaluate LPO because it is a significant product of the degradation of lipid peroxides. The severity of the free radical attack on body cells is indirectly reflected in the level of MDA [21]. In the current study, MDA level in renal tissue of zebrafish elevated altogether upon UV-328 treatment. It can thus be deduced that openness of UV-328 even at low fixations brought about exorbitant age of ROS. Be that as it may, the capacity of the antioxidant prevention agent framework to wipe out ROS was additionally restricted, while the leftover oxygen free radicals

went after the polyunsaturated unsaturated fats in layer, prompting the development of lipid peroxides, i.e., lipid peroxidation, and furthermore an expanded MDA content in tissues. The cell membrane damage caused by lipid peroxidation in zebrafish kidney exposed to UV filters has also been confirmed in previous research [22].

An elevation in MDA level might show tissue injury brought about by oxidative radicals, which may also be reflected by the decrement of antioxidant enzyme markers (e.g., SOD, CAT and GPx movement). The finding of our study claims that tissue MDA level might be an appropriate marker of oxidative stress upon exposure to UV-328. Notwithstanding, as an eventual outcome of lipid peroxidation, MDA increment constantly seen in animals chronically exposed to xenibiotics. MDA contents give more straightforward proof of the toxicants interaction brought about by free radicals than different markers, which can be additionally affirmed by the assurance of ROS levels. In outline, exposure to natural UV filters cannot just prompt the increment of oxidative apprehension in zebrafish kidney, yet additionally lead to lipid peroxidation. DiMC supplemented group showed a superior cell reinforcement status with restricted LPO which obviously involves the antioxidant nature of this phytoconstituent. Dimethoxycurcumin (DiMC), also known as dimethylcurcumin, is a lipophilic compound that is structurally derived from curcumin by converting both of its hydroxyl groups into methoxyl groups [23]. DiMC methylene group in the β-diketone structure gives it striking cell reinforcement properties. In the mean time, the methylation of both hydroxyl groups makes DiMC more stable and lipophilic than curcumin, further furnishing it with a fundamentally decreased degradative rate and a significantly better medicative conveyance framework. It was likewise found that DiMC could act effectively in normal cells in a manner that is like curcumin yet applied stronger cellular antioxidant reinforcement proficiency than curcumin [24].

5. CONCLUSION

The results of the current study open numerous vistas on the impact of organic UV filter on zebrafish. Unanimously numerous natural UV filters have been used in our day today practices as an essential commodity, however it was noticed that a more than adequate amount of UV filters are as yet being delivered, advertised and utilized in different fields all through the world. From our current innovation, it is consistent that when fishes are in immediate or approximate contact with organic UV filter chemicals face an immense loss of its diversity. Numerous biochemical and histological alterations were additionally seen in zebrafish when accessible to sublethal exposure of UV-328 and this multitude of adjustments were considerably recuperated with the supplementation of DiMC due to its powerful antioxidant proficiency. These detections will assist with understanding the spiteful impact of UV filters on fishes and other non-target species and to conquer their unsafe impacts through the intercession with phytoantioxidants similar to DiMC.

ETHICAL APPROVAL

All analyses, maintenance, and treatment dealing with zebrafish were completed according to the rules of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Annamalai University, Tamil Nadu. India. Endorsement number: MB/IAECCC/2022/03/06.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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