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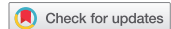
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Assessment of phthalate exposure at a fire site in Korean firefighters

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ABSTRACT

To determine phthalate exposure in 32 firefighters, the concentrations of urinary phthalate metabolites, immediately (exposure day) and three weeks (control day) after fire suppression, were compared. Mono-(2-ethyl-5-carboxypentyl) phthalate, mono-(2-ethyl-5-hydroxyhexyl) phthalate, mono-(2-ethyl-5-oxohexyl) phthalate, mono-n-butyl phthalate (MBP), mono-n-benzyl phthalate (MBzP), and total phthalates (Σ phthalates) levels, and creatinine-adjusted levels of MBP, MBzP, and Σ phthalates were significantly higher on exposure day than on control day. Phthalate concentration was significantly higher in firefighters who performed the fire extinguishing tasks (geometric mean [GM], 149.9 μ g/L) than in those who performed other tasks (GM 70.8 μ g/L) ($p = .012$). The GM concentration of firefighters who were active ≤ 50 m from the fire was 119.0 μ g/L, and 37.6 μ g/L for those who were > 50 m away ($p = .012$). The GM concentration was significantly different ($p = .039$) in firefighters with subjective symptoms after fire suppression (151.9 μ g/L) compared to those without symptoms (81.6 μ g/L). This study showed that firefighters were exposed to phthalate.

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Introduction

Firefighters are exposed to various harmful substances such as polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), particulate matter, and phthalates due to their firefighting activities (Jankovic et al. 1991; Feunekes et al. 1997; Bolstad-Johnson et al. 2000; Ruokojärvi et al. 2000; Austin et al. 2001a, 2001b; Slaughter et al. 2004; Kirk and Logan 2015; Oliveira et al. 2017; Fent et al. 2018; Stec et al. 2018; Kolena et al. 2020). Occupational exposure as a firefighter was previously classified as a possible human carcinogen (Group 2B) by the International Agency for Research on Cancer (IARC) (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2010). However, the IARC re-evaluated firefighting activities and classified occupational exposure of firefighters as carcinogenic to humans based on sufficient evidence for mesothelioma and bladder cancer in humans (Group 1) (Demers et al. 2022).

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Existing research on exposure of firefighters to hazardous substances has mainly focused on carcinogenic substances; however, there are increasing interests in the exposure of firefighters to noncarcinogens. Firefighters are exposed to various endocrine-disrupting substances, including phthalates, from firefighting activities (Poutasse et al. 2022). Phthalates have been reported to be associated with various health effects such as cardiovascular diseases, respiratory diseases, thyroid diseases, diabetes, obesity, kidney diseases, intelligence performance in children and reproductive system-related diseases (Fu et al. 2020; Sears and Braun 2020). Among these health effects, decrease in sperm quality in males and attention-deficit/hyperactivity disorder in children have been consistently reported (Chang et al. 2021). Phthalates are used in various items such as personal care products, plastics, paints, and some medical devices and pharmaceuticals (Chou and Wright 2006; Romero-Franco et al. 2011; Kelley et al. 2012; Nassan et al. 2017; Fisher et al. 2019). With the increasing use of plastic products, firefighters are increasingly being exposed to phthalates at fire sites.

Phthalates' exposure in firefighters can occur via inhalation, dermal absorption, and ingestion. Biomonitoring is a useful method for evaluating exposure because it can integrate multiple routes of exposure and directly reflect the total body burden. Various biological fluids, including blood, urine, saliva, and respiratory fluids, are used in biomonitoring, and urine is the most common and least invasive matrix for evaluating occupational biomarkers of exposure. The assessment of human exposure to phthalates is based on the measurement of its monoester metabolites in urine. Phthalates have a short half-life (2–12 h) in the body and are rapidly excreted in urine as monoester metabolites (Calafat and McKee 2006). Thus, measurement of urine phthalate metabolites are suitable biomarkers for assessing human exposure to the parent compounds (Silva et al. 2003). Over the past 25 years (1995–2020), there have been 44 studies on occupational exposure of firefighters through the assessment of urinary biomarkers, most of which focused on PAHs and heavy metals (Barros et al. 2021). Although there have been concerns about firefighters' exposure to phthalates, few studies have measured phthalate levels in firefighters due to difficulties in predicting fire outbreaks as well as difficulties in assessment due to the short half-life of phthalates (less than 24 hours). This study aimed to evaluate phthalate exposure in firefighters after a fire event and on a normal day using biomonitoring.

Material and methods

This study was approved by the institutional review board of Wonju Severance Christian Hospital (CR320127). The researchers explained the purpose, methods, and risks of the study to the study participants and obtained their informed consent.

Study participants

The participants of this study were 32 firefighters who were involved in extinguishing a fire, at a large fire breakout site for more than two hours on 26 September 2020 at an Auto Parts Factory, which was extinguished in approximately four hours. To compare the difference before and after fire suppression, the required sample size was calculated using G-power 3.1 (Faul et al. 2007). The appropriate sample sizes were 34 and 27, respectively, for two-tailed and one-sided paired *t*-test with an α error of 0.05, a power of 80%, and an effect size of 0.5.

Urine sample collection and survey

The firefighters' urine samples were collected immediately after the fire was extinguished (exposure day) and three weeks later (control day) if they had not participated in any other firefighting events for ≥ 2 h during this period (hereinafter referred to as "normal"). The urine samples were stored appropriately till they were used for analysis. Surveys were conducted with questionnaires two times, on exposure and control day, respectively. The first questionnaire, on

exposure day, included information regarding main work at the fire site, distance from the fire site, time spent at the fire site, types of respirators and wearing time, and whether they thought they were exposed to chemicals during firefighting. On control day, clinical symptoms were surveyed with the second questionnaire, which included details of the oropharyngeal system, ophthalmic system, respiratory system, skin, neuropsychiatric system, nervous system, cardiovascular system, oral cavity and teeth, and digestive system, within seven days of extinguishing the fire.

Sample preparation

Each 2-mL urine sample was thawed in a 15-mL test tube. The sample was spiked with the labeled internal standard solution (20 μ L, 1 μ g/mL), 1 M ammonium acetate (100 μ L), and β -glucuronidase (10 μ L, 200 U/mL). Mono(3-carboxypropyl)phthalate-d4 (MCPD-d4) (Toronto Research Chemicals inc, Canada) and Mono butyl phthalate-d4 (MBP-d4) (Toronto Research Chemicals inc, Canada) were used as internal standards. The samples were then incubated at 37°C for 3 h in a drying oven. The prepared sample and 1 ml methanol were mixed, put into a syringe (HENKE-JECT 1 mL, HENKE SASS WOLF*), and filtered with a syringe filter (0.2 μ m pore size, 13 mm diameter, 13HP020AN, ADVANTEC*).

LC-MS/MS analysis of urinary phthalate metabolites

Eight metabolites [mono-n-butyl phthalate (MBP), mono-benzyl phthalate (MBzP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-(carboxyisooctyl) phthalate (MCiOP), mono-(carboxyisononyl) phthalate (MCiNP), mono-(3-carboxypropyl) phthalate (MCPD)] for six phthalate source substances [dibutyl phthalate (DBP), benzylbutyl phthalate (BzBP), di-2-ethylhexyl phthalate (DEHP), diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP), di-n-octyl phthalate (DnOP)] were quantified in urine (Supplementary table S1). Urinary phthalate metabolites were identified and quantified using a Dionex UltiMate 3000 ultrahigh-performance liquid chromatography (UHPLC) system (Thermo Fisher Scientific, Waltham, MA) equipped with a TSQ Quantis tandem mass spectrometer (MS/MS) with negative electrospray ionization. Separation was performed on a ZORBAX SB-Phenyl HPLC column (2.1 \times 50 mm, 1.8 μ m; Agilent Technologies, Santa Clara, CA). The column temperature was maintained at 4°C. The mobile phases were as follows: A, 0.1% acetic acid in deionized water; B, 0.1% acetic acid in methanol. Graduated elution of the mobile phases was performed as follows: 0–10 min, 30–40% B; 10–14 min, 40–60% B; 14–19.8 min, 60% B; 19.8–27 min, 60–100% B; 27–28.5 min, 100% B; 28.5–30 min, 100–30% B; 30–33 min, 30% B. The injection volume was 10 μ L, and the flow rate was set to 0.2 mL/min. MS/MS parameters were as follows: capillary voltage, 3.0 kV; gas temperature, 350°C; gas flow (nitrogen) 7.97 L/min. The mass spectrometric parameters and limits of detection (LOD) are listed in Supplementary table 2.

Quality control for the analyses of phthalate metabolites used a calibration curve of samples made of standard substances. After preparation with the addition of an internal standards to the urine samples, the final concentrations were calculated by substituting the ratio between the peak area of the final metabolites and the peak area of the internal standards into the calibration equation.

Statistical analyses

When the concentrations of phthalate metabolites were <LOD, the values were replaced with a value obtained by dividing LOD by square root of 2. As the data were not normally distributed according to the Shapiro – Wilk and Kolmogorov – Smirnov tests, the geometric mean (GM) and geometric standard deviation (GSD) of the urine concentrations were presented. The paired *t*-test for metabolites with normal distribution after logarithmic transformation was used to compare phthalate metabolite

levels between the exposure and control days. Wilcoxon rank-sum test was used for other variables. The Mann – Whitney U test was used for comparison according to the working conditions at the fire site. SPSS (v25.0.0; IBM, Armonk, NY) was used for statistical analyses.

Results

General characteristics of participants and working conditions of fire sites

All 32 firefighters participating in the study were male, with an average age of 39.3 ± 8.5 years. Table 1 presents the survey results. The most common task at the fire site was fire suppression ($n = 16$, 50%), followed by rescue ($n = 8$, 25%) and driving ($n = 7$, 21.9%). The distance between the fire site and the place where the person worked were, ≤ 50 m for 28 (87.5%); 50–100 m for 2 (6.3%) and >100 m for 2 firefighters, respectively. Most firefighters ($n = 15$, 46.9%) stayed at the fire site for 4–6 h, followed by 7 (21.9%) for 2–4 h, and 5 (15.6%) for 6–8 h. Within 7 days of extinguishing the fire, 12 firefighters (37.5%) had symptoms in the oropharyngeal system, ophthalmic system, respiratory system, skin, neuropsychiatric system, nervous system, cardiovascular system, oral cavity, teeth, and digestive system.

Urinary phthalate metabolites on exposure and control days

On exposure day, MEHHP, MEOHP, MECPP, MBP, and MBzP were detected in all participants, MCiNP in 28 (91%), MCPP in 15 (47%), and MCiOP in 14 (44%). On control day, MEHHP, MEOHP, MECPP, and MBP were detected in all participants, MBzP in 29 (91%), MCiNP in 25 (78%), MCPP in 10 (31%), and MCiOP in 5 (16%).

The concentration of MBP was significantly higher on exposure day than on control day ($GM \pm GSD$, 58.1 ± 3.05 vs. 19.3 ± 2.49 $\mu\text{g/L}$; $p < .001$), even after adjusting for creatinine ($GM \pm GSD$, 22.9 ± 2.64 vs. 10.4 ± 2.38 $\mu\text{g/g creatinine}$; $p < .001$). Similarly, the mean \pm standard deviation (SD) concentration of MBzP was significantly higher on exposure day than on control day (2.018 ± 2.472 vs. 0.567 ± 4.393 $\mu\text{g/L}$; $p < .001$), even after adjusting for creatinine (mean \pm SD, 1.118 ± 0.933 vs. 0.558 ± 0.547 $\mu\text{g/g creatinine}$; $p = .004$). The concentrations of DEPH-MEHHP, MEOHP, and MECPP were also significantly higher on exposure than on control day, but there were no significant differences after creatinine adjustment. Further, the concentration of total phthalates was significantly higher on exposure day than on control day ($GM \pm GSD$, 103.0 ± 2.39 vs. 45.8 ± 2.42 $\mu\text{g/L}$; $p < .001$), even after creatinine adjustment (40.6 ± 2.10 vs. 24.7 ± 2.39 $\mu\text{g/g creatinine}$; $p = .010$; Table 2).

Table 1. General characteristics of the participants.

Classification		N	%
Work at the fire site	Fire extinguishing	16	50.0
	Rescue	8	25.0
	Driving	7	21.9
	Rescue and cleanup	1	3.1
Distance from the fire site	≤ 50 m	28	87.5
	50–100 m	2	6.3
	>100 m	2	6.3
Time spent at the fire site	<2 h	2	6.3
	2–4 h	7	21.9
	4–6 h	15	46.9
	6–8 h	5	15.6
	>8 h	3	9.4
Symptoms within 7 days of working at the fire site	Yes	12	37.5
	No	20	62.5

Table 2. Concentration of urinary phthalate metabolites on exposure and control days.

Phthalate metabolites	Creatinine-unadjusted ($\mu\text{g/L}$)					Creatinine-adjusted ($\mu\text{g/g Cr}$)				
	Exposure		Control		<i>p</i> -value	Exposure		Control		<i>p</i> -value
	GM	GSD	GM	GSD		GM	GSD	GM	GSD	
MEHHP	14.3	1.94	9.8	2.48	.035	5.65	1.841	5.30	2.506	0.698
MEOHP	4.5	1.91	3.1	2.53	.040	1.77	1.843	1.667	2.556	0.716
MECPP	14.0	1.98	9.5	2.60	<.001	5.51	1.947	5.15	2.593	0.696
MBP	58.1	3.05	19.3	2.49	<.001	22.89	2.640	10.43	2.375	<0.001
MBzP*	2.018	2.472	0.567	4.393	<.001	1.118	0.933	0.558	0.547	0.004
MCiOP*	0.050	2.922	0.033	2.807	0.144	0.033	0.040	0.041	0.077	0.405
MCP*P*	0.108	7.352	0.055	5.348	0.117	0.367	1.261	0.261	0.995	0.360
MCiNP*	0.032	3.470	0.022	4.142	0.258	0.021	0.018	0.021	0.020	0.844
Σ Phthalates	103.0	2.39	45.8	2.42	<.001	40.623	2.103	24.703	2.393	0.010

GM: geometric mean, GSD: geometric standard deviation, MEHHP: mono-(2-ethyl-5-hydroxyhexyl) phthalate, MEOHP: mono-(2-ethyl-5-oxohexyl) phthalate, MECPP: mono-(2-ethyl-5-carboxypentyl) phthalate, MBP: mono-n-butyl phthalate, MBzP: mono-benzyl phthalate, MCiOP: mono-(carboxyisooctyl) phthalate, MCP*P*: mono-(3-carboxypropyl) phthalate, MCiNP: mono-(carboxyisononyl) phthalate, Σ Phthalates = sum of eight metabolites quantified.

*Wilcoxon signed rank test, each value is mean and standard deviation.

Table 3. Concentration of urinary phthalate metabolites according to the working conditions at the fire site.

Classification	N	Creatinine-unadjusted			Creatinine-adjusted			
		Σ Phthalates ($\mu\text{g/L}$)		<i>p</i> -value	Σ Phthalates ($\mu\text{g/g Cr}$)		<i>p</i> -value	
		GM	GSD		GM	GSD		
Work at the fire site	Fire extinguishing	16	149.9	2.48	0.012	54.6	2.19	0.022
	Other tasks	16	70.8	1.94		30.2	1.80	
Distance from the fire site	≤ 50 m	28	119.0	2.25	0.012	44.8	2.05	0.040
	> 50 m	4	37.6	1.77		20.5	1.83	
Time spent at the fire site	> 6 h	8	110.9	2.72	0.223	28.8	2.33	0.107
	< 6 h	24	74.9	2.27		45.6	1.99	
Symptoms within 7 days of working on the fire site	Yes	12	151.9	2.39	0.039	63.1	2.04	0.010
	No	20	81.6	2.23		31.2	1.90	

GM:geometric mean, GSD: geometric standard deviation.

Urinary total phthalates according to the working conditions at the fire site

The concentration of urinary phthalates was significantly higher in firefighters who were directly involved in fire extinguishing tasks than in those who performed other tasks (GM \pm GSD, 149.9 ± 2.48 vs. 70.8 ± 1.94 $\mu\text{g/L}$; $p = .012$), even after creatinine adjustment (GM \pm GSD, 54.6 ± 2.19 vs. 30.2 ± 1.80 $\mu\text{g/g creatinine}$; $p = .022$). Further, the concentration of urinary phthalates was significantly higher in firefighters who worked ≤ 50 m than in those who worked > 50 m from the fire site (GM \pm GSD, 119.0 ± 2.25 vs. 37.6 ± 1.77 $\mu\text{g/L}$; $p = .012$), even after creatinine adjustment (GM \pm GSD, 44.8 ± 2.05 vs. 20.5 ± 1.83 $\mu\text{g/g creatinine}$; $p = .040$). Moreover, the concentration of urinary phthalates was significantly higher in firefighters who experienced symptoms within seven days after extinguishing the fire than in those without symptoms (GM \pm GSD, 151.9 ± 2.39 vs. 81.7 ± 2.23 $\mu\text{g/L}$; $p = .039$), even after creatinine adjustment (GM \pm GSD, 63.1 ± 2.04 $\mu\text{g/g}$ vs. 31.2 ± 1.90 $\mu\text{g/g creatinine}$; $p = .010$). There were no differences in the concentration of urinary phthalates according to time at the fire site (Table 3).

Discussion

This study showed that urinary phthalate metabolites increased following firefighting event in firefighters, particularly in those who are involved directly in extinguishing the fire and who are closer to the fire. The concentration of phthalate metabolites in urine was significantly higher in those with clinical symptoms after fire suppression than in those without clinical symptoms. Because firefighters

are exposed to complex mixtures of pollutants, the higher levels of phthalate metabolites in urine in those with clinical symptoms after fire suppression may be due to the firefighter also exposed to other chemicals (e.g. some VOCs, or flame retardants) which can cause the similar clinical symptoms.

Firefighters are exposed to various hazardous substances due to combustion during firefighting activities; however, it is difficult to evaluate firefighters' exposure because fires occur unexpectedly. Most previous studies on firefighters have measured the concentration of harmful substances in the atmosphere at fire sites (Fent et al. 2018). Although exposure evaluation studies using new devices are being conducted on firefighters, external measurements do not reflect internal levels (Poutasse et al. 2020, 2022; Levasseur et al. 2022).

Phthalates are widely used as plasticizers and additives in polyvinyl chloride (PVC) plastics and personal care products (Schettler 2006). They are ubiquitous contaminants in the environment, and firefighters can be exposed to them through combustion or equipment such as fire extinguishing foams or protective gear (Lacey et al. 2014; Stevenson et al. 2015). Among the eight phthalate metabolites quantified in the urine, the three metabolites of DEHP – MEHHP, MEOHP, and MECPP; MBP, a metabolite of DBP; and MBzP, a metabolite of BzBP, increased significantly (Silva et al. 2007). DEHP, DBP, and BzBP are used as plasticizers in plastics (Gimeno et al. 2014). The substances to which firefighters are exposed depends on the raw material that is burned. The increased levels of these substances are thought to be because of the exposure of the participants in this study was a fire that occurred in an automobile part factory which was made of sandwich panels with expanded polystyrene and urethane fillings.

In the Firefighter Occupational Exposures (FOX) Project, a firefighter cohort in 2010–2011, the concentrations of urine phthalate metabolites MECPP and MBP were 12.3 and 10.6 µg/L, respectively, lower than those on exposure day and higher than those on control day in this study and MBzP and MCPPE were higher at 8.18 µg/L and 1.88 µg/L, respectively. In a study by Kolena et al. (2020) on Slovakian firefighters, MEHHP and MBP were 13.33 µg/L and 56.26 µg/L, respectively, which were also lower than concentrations after fire suppression and higher than at control day in this study. MEOHP and MECPP were higher at 7.86 µg/L and 14.63 µg/L, respectively. Many biomonitoring studies have been conducted in firefighters (Barros et al. 2021). However, limited studies have evaluated phthalate exposure in firefighters and different evaluation methods such as sampling time and metabolites have been used, making it difficult to evaluate phthalate exposure in firefighters. In this study, the concentrations of urinary phthalate metabolites were measured directly in urine immediately after fire suppression and compared to those on control day.

Urine concentrations of seven phthalate metabolites except MCPPE decreased after creatinine correction. MEHHP, MEOHP, and MECPP concentrations, which were statistically significantly higher after fire suppression, showed no significant difference after creatinine correction. The urinary concentration of metabolites is affected by the rate of urine production. Therefore, urinary creatinine correction has been used to exclude samples that are too dilute or too concentrated in biological monitoring (World Health Organization, 1996). However, creatinine itself is not excreted at a constant rate, and hence, it is not suitable in untimed samples such as spot urine samples (Boeniger et al. 1993). Firefighters at fire sites are prone to dehydration in high-temperature environments with high physical workload. Therefore, the urine sample in this study collected after fire suppression may also be over-concentrated. However, for these reasons, creatinine-unadjusted concentrations may be interpreted as reflecting actual exposure levels in firefighters. Therefore, we compared both unadjusted and creatinine-adjusted concentrations.

The group with clinical symptoms exhibited a significantly higher concentration of phthalate metabolites in their urine. In a study by Witt et al. (2017), firefighters were found to have a statistically higher prevalence of symptoms such as morning cough, daytime or nighttime coughing, morning expectoration, shortness of breath, wheezing, and nocturnal breathlessness attacks. These symptoms were found to be associated with the duration of occupational exposure. Kolena et al. (2020)

reported that phthalate metabolites in the urine of Slovakian firefighters were linked to pulmonary function, specifically the forced expiratory volume in 1 second (FEV1). However, it should be noted that this association does not necessarily indicate a causal relationship. Bessonneau et al. (2021) reported that exogenous exposure to phthalates can impact health outcomes by interacting with endogenous metabolites.

Phthalates, which are endocrine-disrupting chemicals, have been reported to be associated with various health effects such as reproductive system diseases, cardiovascular diseases, thyroid disorders, respiratory diseases, diabetes, obesity, kidney diseases, and neurological disorders (Mariana et al. 2016; Wang et al. 2019; Fu et al. 2020; Sears and Braun 2020; Chang et al. 2021). However, consistent associations have been reported regarding decreased sperm quality in males and ADHD-related behavior problems in children (Chang et al. 2021). While further research is needed to fully understand the health effects of phthalates, it is anticipated that prolonged exposure to phthalates among firefighters could increase the potential risk of adverse health outcomes. While previous research has primarily focused on carcinogenic substances in firefighting, this study emphasizes the significance of evaluating exposure to noncarcinogens such as phthalates.

Exposure assessment studies using novel biomonitoring approaches have been conducted in firefighters exposed to various substances (Grashow et al. 2020). Because firefighters are exposed to complex mixtures of pollutants and the level exposure is determined by the length of exposure, the type of combustion products, and concentration of the pollutants, an increase in biomarkers of exposure may indicate the possibility of exposure of firefighters to phthalates as well as various other hazardous substances (Fent et al. 2018; Sjöström et al. 2019). Efforts to reduce exposure and conduct precise exposure assessments should be made.

This study has several limitations. This study focused on evaluating acute exposure indicators and health effects of short-term fire emission exposure in firefighters. However, actual firefighter exposure occurs repeatedly over a long period. Therefore, longitudinal studies are necessary to understand the long-term health effects of chronic exposure on firefighters. Additionally, this study primarily examined biomarkers of exposure rather than biomarkers of effects. Further studies investigating biomarkers of effects, rather than just exposure, are needed for a better understanding of the potential health risks of fire emission exposure in firefighters. Furthermore, this study was conducted during a fire at an automotive parts factory. Since the types of hazardous substances that firefighters are exposed to can vary depending on the materials involved in the fire, future research should investigate hazardous substances exposed at various fire sites to gain a more comprehensive understanding.

Conclusion

Firefighters are essential members of the public service profession. This study confirmed that firefighters are exposed to phthalates during fire suppression. Further research is needed to better evaluate firefighters' occupational safety and health risks, and to implement preventive strategies.

Disclosure statement

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