

Association between drinking water fluoride and the serum alkaline phosphatase and phosphate levels in pregnant women and newborn infants

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D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online)

Dent Med Probl. 2023;60(4):569–575

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Funding sources

Indian Council of Medical Research (ICMR), New Delhi, India (5/9/1158/2015-NUT).

Conflict of interest

None declared

Acknowledgements

None declared

Received on November 7, 2020

Reviewed on January 17, 2021

Accepted on January 22, 2021

Published online on August 1, 2023

Cite as

Honne Manjunathappa T, Devegowda D, Mysore NK, Vishwanath P, Sathya Narayana P. Association between drinking water fluoride and the serum alkaline phosphatase and phosphate levels in pregnant women and newborn infants. *Dent Med Probl.* 2023;60(4):569–575. doi:10.17219/dmp/132692

DOI

10.17219/dmp/132692

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Abstract

Background. Endemic fluorosis (skeletal and dental) is a serious public health problem in many parts of the world, especially in India. Age, sex, dietary calcium (Ca), the hormonal status, the dose and duration of the fluoride intake, and renal efficiency in handling fluoride all influence fluoride metabolism.

Objectives. The aim of the study was to evaluate the effect of the fluoride present in drinking water on the serum alkaline phosphatase (ALP) and phosphate levels in pregnant women and newborn infants.

Material and methods. In the present cross-sectional study, the participants were categorized into 2 groups based on a fluoride concentration in their drinking water: the low/optimum-fluoride group (<1 ppm); and the high-fluoride group (≥1 ppm). Each group was comprised of 90 pregnant women who were recruited from the hospital at the time of admission for delivery. Fluoride was measured in their drinking water, urine, maternal serum, and cord blood. The ALP and phosphate levels were measured in serum using a fully automated analyzer.

Results. The drinking water consumed by the pregnant women contained fluoride, which was significantly positively correlated with the urine and blood serum fluoride levels. There were significant differences in the ALP levels between the 2 groups in both maternal serum and cord blood. The level of phosphate in maternal serum was significantly higher in the high-fluoride group. The results of both simple and multi-variate regression analyses revealed that the fluoride content in drinking water was significantly associated with the ALP level in cord blood and the phosphate level in maternal serum.

Conclusions. The ALP levels were negatively associated with drinking water fluoride concentrations in both maternal serum and cord blood. The phosphate levels in maternal serum were positively associated with drinking water fluoride concentrations.

Keywords: pregnancy, cord blood, alkaline phosphatase, fluoride, serum phosphate

Introduction

Fluorine (F) is the 13th most common element in the Earth's crust, and is widely distributed as fluorspar, fluorapatite and cryolite. These minerals are easily soluble in water and are present in groundwater, which contains high levels of fluoride.¹ The public health benefits associated with fluoridated dental products and optimally fluoridated drinking water are widely cited.² Besides the benefits, long-term fluoride exposure can also cause adverse effects, such as dental fluorosis and skeletal fluorosis.² Endemic fluorosis (skeletal and dental) is a serious public health problem in many parts of the world, especially in India. Age, sex, dietary calcium (Ca), the hormonal status, the dose and duration of the fluoride intake, and renal efficiency in handling fluoride all influence fluoride metabolism.³ Fluoride is known to cross the placental barrier. In vitro studies have shown that it accumulates in brain regions involved in learning and memory, and alters proteins and neurotransmitters in the central nervous system (CNS).⁴ Hence, it can be presumed that fluoride can also adversely affect CNS.

Alkaline phosphatase (ALP) is an enzyme that occurs in all tissues of the human body. A high concentration of ALP is found in bone, liver, kidney, intestinal, and placental tissue. During pregnancy, ALP is known to gradually increase, reaching a peak in the 3rd trimester that is approx. twice its pregestational value.⁵ Serum bone-specific ALP is one of the most specific markers of bone formation.⁶

Phosphorus (P) is important in DNA synthesis and acts as a mitogen. Dietary P deficiency is rare, since most foods contain this element. The recommended dietary allowance (RDA) for P is 700 mg/day for both pregnant and non-pregnant women, and no additional supplementation is recommended for pregnant women based on the current knowledge.⁷

A literature search revealed no studies associating fluoride in drinking water with pregnant women's serum ALP and phosphate levels. Therefore, the present study hypothesized that the fluoride present in drinking water is associated with the serum ALP and phosphate levels in pregnant women.

Methodology

Based on a fluoride concentration in their drinking water, pregnant women were categorized into 2 groups. One group was considered low/optimum-fluoride (concentration below 1 ppm) and the other group was considered high-fluoride (concentration equal to or above 1 ppm) based on the World Health Organization (WHO) criteria.⁸ Ninety pregnant women from each group were included in the study. Before the commencement of the study, ethical clearance was obtained from the institutional ethics committee at the JSS Dental College and Hospital, Mysore, India (No. JSS/DC/Ethical/2014-15).

All subjects were recruited from the Department of Gynecology of the JSS Hospital, Mysore, India, during a prenatal visit approx. 1 month prior to their due date. Subjects who provided written informed consent were included in the study. Data on any prediagnosed endocrine disorders, serious pregnancy complications and/or bone disorders was obtained from medical records. If any of these conditions were present, the subject was excluded from the study. All data collection was performed during 1 year (July 2019–June 2020). At the time of recruitment, the mother's age, socioeconomic status (SES), educational level, and medical history, as well as the type of drinking water, were recorded. The socioeconomic status was categorized based on the modified version of the Kuppuswamy classification.⁹

The sample size was calculated based on a power of 80% and a 95% confidence interval (CI) according to previous study findings, assuming a mean difference (MD) of 40 and a standard deviation (SD) of 92 in the ALP levels. The records pertaining to the infant's length, weight and head circumference were assessed by a well-trained staff member using a standardized protocol. The duration of the pregnancy was also recorded.

Fluoride analysis

The study participants were asked to obtain samples of the water they consumed during the course of their pregnancy. A fluoride concentration in drinking water, urine, maternal serum, and cord blood was assessed according to the American Public Health Association (APHA) guidelines, using a 9609BNWP fluoride electrode (Orion™; Thermo Scientific, Mumbai, India). The electrode was calibrated daily before it was used to measure the fluoride concentration. Fasting urine and serum samples before delivery, and cord blood after delivery were used to assess fluoride concentrations.

Laboratory measurements

First, the blood samples collected from the pregnant women for routine investigation before delivery were used to assess the fluoride, ALP and phosphate levels. Then, after delivery, these parameters were assessed in the cord blood samples. The blood samples were collected and immediately placed in tubes, labeled and submitted to the hospital's clinical analysis laboratory, where they were centrifuged and transported under refrigeration. The ALP and phosphate levels were analyzed using a fully automated chemistry analyzer (TBA-120FR™; Toshiba, Tokyo, Japan). All samples were stored at –20°C.

Data management and statistical analysis

All the collected data was entered into a Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, USA).

The IBM SPSS Statistics for Windows, v. 23.0 (IBM Corp., Armonk, USA), was used for data analysis. For descriptive data analysis, mean and standard deviation ($M \pm SD$) or frequency and percentage (n (%)) were used. For inferential data analysis, the unpaired two-sample t test was used to compare data with normal distribution, and the non-parametric Mann–Whitney U test was used to compare data with skewed distribution. The normality of data was tested using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The frequencies and percentages were compared using Pearson's χ^2 test. Associations for both groups were determined using Spearman's correlation test.

To determine the one-to-one association, a simple linear regression model was used. In the model, the drinking water fluoride content was considered an independent variable, and the serum ALP and phosphate levels were entered as dependent variables. After the consideration of covariates, a multivariate linear regression analysis was performed. Covariates that showed significant differences between the low/optimum-fluoride and high-fluoride groups in inferential statistics were considered covariates in the multivariate regression analysis. The covariates which exhibited significant differences were SES and maternal education. The multivariate regression analysis model equation was used to predict the serum ALP and phosphate levels, with an increase by 1 ppm of fluoride in drinking water. A p -value <0.05 was considered statistically significant.

Results

There were 2 groups examined. One group had a low/optimum fluoride concentration in their drinking water (0.50 ± 0.28 ppm) and the other had a high fluoride concentration in their drinking water (2.65 ± 1.29 ppm). The mean age of the pregnant women in the low/optimum-fluoride group was 23.88 ± 3.57 years, while it was 24.13 ± 3.85 years in the high-fluoride group. Other baseline characteristics considered were SES, maternal education, the duration of pregnancy, the maternal height, weight and body mass index (BMI), and the birth weight of the newborn. The 2 groups differed significantly in terms of SES and maternal education. Fluoride concentrations in urine, maternal serum and cord blood were 0.200 ± 0.240 ppm, 0.014 ± 0.014 ppm and 0.011 ± 0.011 ppm in the low/optimum-fluoride group, and 1.920 ± 1.190 ppm, 0.153 ± 0.113 ppm and 0.110 ± 0.100 ppm in the high-fluoride group, respectively. The comparison of the values of all parameters between the low/optimum-fluoride and high-fluoride groups showed statistically significant differences ($p < 0.001$) (Table 1).

Table 2 presents the serum ALP and phosphate levels in the 2 groups. Significantly lower levels of ALP were observed in both maternal serum and cord blood in the high-fluoride group. Conversely, the levels of phosphate in maternal serum were significantly higher in the high-fluoride group as compared to the low/optimum-fluoride group.

Table 1. Baseline demographic and clinical characteristics of the study population

Variable	Group		p -value
	low/optimum-fluoride $n = 90$	high-fluoride $n = 90$	
Age [years]	23.88 ± 3.57	24.13 ± 3.85	0.645
SES	class I	13 (14.4)	3 (3.3)
	class II	26 (28.9)	18 (20.0)
	class III	31 (34.4)	23 (25.6)
	class IV	15 (16.7)	27 (30.0)
	class V	5 (5.6)	19 (21.1)
Maternal education	illiterate	4 (4.4)	10 (11.1)
	primary (1–4 years)	10 (11.1)	23 (25.6)
	middle (5–7 years)	16 (17.8)	26 (28.9)
	high school and PUC	32 (35.6)	23 (25.6)
	degree and diploma	28 (31.1)	8 (8.9)
Maternal height [ft]	5.14 ± 0.98	5.15 ± 0.87	0.763
Maternal weight [kg]	58.26 ± 4.26	57.39 ± 4.39	0.253
BMI [kg/m^2]	23.87 ± 2.79	23.48 ± 2.67	0.282
Duration of pregnancy [weeks]	38.31 ± 1.13	38.47 ± 0.97	0.321
Birth weight of the newborn [kg]	2.69 ± 0.57	2.60 ± 0.56	0.274
Drinking water fluoride concentration [ppm]	0.500 ± 0.280	2.650 ± 1.290	0.000**
Urine fluoride concentration [ppm]	0.200 ± 0.240	1.920 ± 1.190	0.000**
Maternal serum fluoride concentration [ppm]	0.014 ± 0.014	0.153 ± 0.113	0.000**
Cord blood fluoride concentration [ppm]	0.011 ± 0.011	0.110 ± 0.100	0.000**

Data presented as mean $\pm$ standard deviation ($M \pm SD$) or as frequency (percentage) (n (%)). SES – socioeconomic status; BMI – body mass index; PUC – pre-university course; ** highly statistically significant.

Table 3 shows the strength of the association between the amount of fluoride present in drinking water, urine, maternal serum, and cord blood and the serum ALP and phosphate levels. The ALP levels were negatively associated with fluoride concentrations, while the maternal serum phosphate levels were positively associated with fluoride concentrations (Fig. 1,2).

Table 4 presents the results of the simple linear regression analysis and the selected covariates for the multivariate regression analysis. In the simple linear regression analysis, the serum ALP and phosphate levels were considered dependent variables, and the drinking water fluoride concentration was considered an independent variable. In the multivariate regression analysis, SES and maternal education were considered. The results of both simple and multivariate regression analyses revealed that the fluoride content in drinking water was significantly associated with the ALP level in cord blood and the phosphate level in maternal serum.

Discussion

This study was designed to determine fluoride concentrations in pregnant mothers' drinking water, urine and blood before delivery, and in cord blood after delivery. Another objective of the study was to investigate the associations between the fluoride content and the ALP and phosphate levels in maternal serum right before delivery and in cord blood after delivery. To the best of our knowledge, this is the first study to evaluate the effects of fluoride on the aforementioned parameters at both low/optimum and high levels of fluoride in the drinking water consumed by pregnant women.

This study investigated the association between the urine and serum fluoride levels in pregnant mothers right before delivery and the cord blood fluoride levels after delivery. The findings of the study reveal that as the fluoride concentration in drinking water increased,

Table 2. Comparison of the serum alkaline phosphatase (ALP) and phosphate levels in pregnant women consuming low/optimum-fluoride and high-fluoride drinking water

Variable			Group		p-value
			low/optimum-fluoride n = 90	high-fluoride n = 90	
ALP [IU/L]	maternal serum		326.78 ± 135.69	279.72 ± 136.89	0.018*
	cord blood		254.14 ± 121.95	195.79 ± 107.94	0.001**
Phosphate [mg/dL]	maternal serum		3.47 ± 2.65	4.47 ± 3.43	0.001**
	cord blood		4.49 ± 2.98	4.12 ± 2.26	0.285
ALP [IU/L]	maternal serum	<37 (deficient)	0 (0.0)	0 (0.0)	0.130
		37–306 (normal)	47 (52.2)	58 (64.4)	
		>306 (excess)	43 (47.8)	32 (35.6)	
	cord blood	<37 (deficient)	0 (0.0)	0 (0.0)	0.009*
		37–306 (normal)	64 (71.1)	79 (87.8)	
		>306 (excess)	26 (28.9)	11 (12.2)	
Phosphate [mg/dL]	maternal serum	<2.8 (deficient)	33 (36.7)	11 (12.2)	0.001**
		2.8–4 (normal)	47 (52.2)	53 (58.9)	
		>4 (excess)	10 (11.1)	26 (28.9)	
	cord blood	<2.8 (deficient)	18 (20.0)	21 (23.3)	0.734
		2.8–4 (normal)	31 (34.4)	33 (36.7)	
		>4 (excess)	41 (45.6)	36 (40.0)	

Data presented as $M \pm SD$ or as n (%). * statistically significant; ** highly statistically significant.

Table 3. Spearman's correlation between fluoride concentrations in drinking water, urine, maternal serum, and cord blood and the alkaline phosphatase (ALP) and phosphate levels in maternal serum and cord blood

Correlation	ALP in maternal serum	ALP in cord blood	Phosphate in maternal serum	Phosphate in cord blood
Drinking water fluoride concentration	−0.148 ($p < 0.048^*$)	−0.248 ($p < 0.001^{**}$)	0.287 ($p < 0.000^{**}$)	−0.043 ($p < 0.571$)
Urinary fluoride concentration	−0.113 ($p < 0.131$)	−0.207 ($p < 0.005^{**}$)	0.260 ($p < 0.000^{**}$)	−0.042 ($p < 0.574$)
Maternal serum fluoride concentration	−0.187 ($p < 0.012^*$)	−0.300 ($p < 0.000^{**}$)	0.236 ($p < 0.001^{**}$)	−0.104 ($p < 0.165$)
Cord blood fluoride concentration	−0.159 ($p < 0.033^*$)	−0.313 ($p < 0.000^{**}$)	0.175 ($p < 0.019^*$)	−0.148 ($p < 0.470$)

* statistically significant; ** highly statistically significant.

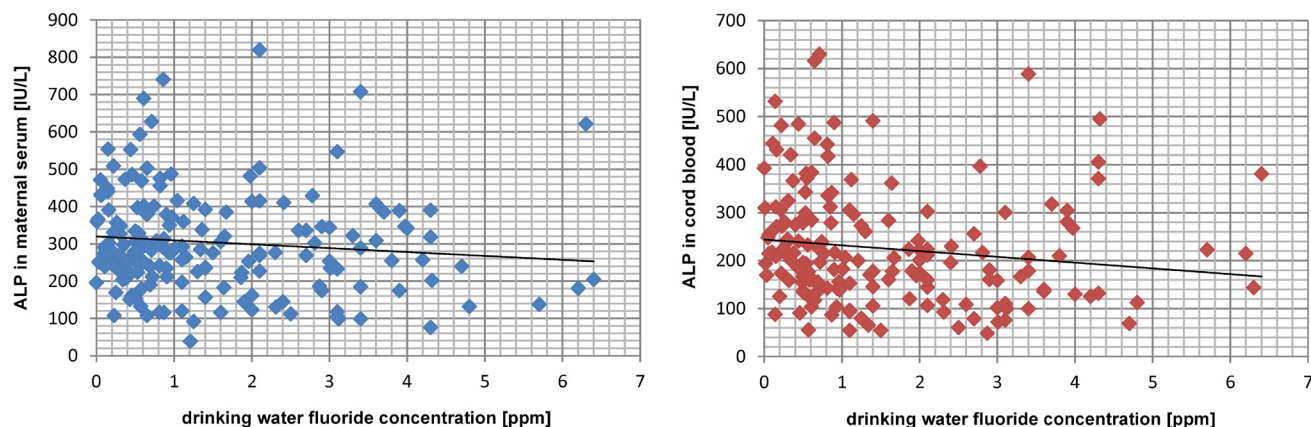


Fig. 1. Correlation between the drinking water fluoride concentration and the alkaline phosphatase (ALP) level in maternal serum and cord blood

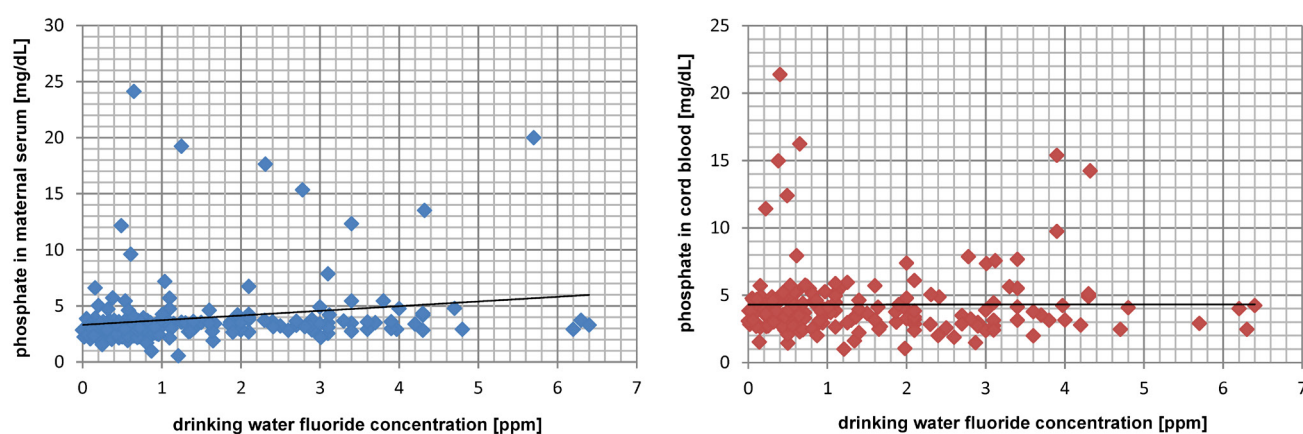


Fig. 2. Correlation between the drinking water fluoride concentration and the phosphate level in maternal serum and cord blood

Table 4. Unadjusted and adjusted associations estimated based on the linear regression models between the drinking water fluoride concentration and the alkaline phosphatase (ALP) and phosphate levels in maternal serum and cord blood

Parameter			Constant	B	SE	p-value
ALP	maternal serum	†	319.664	−10.403	7.189	0.150
		‡	300.318	−9.620	7.621	0.208
	cord blood	†	243.936	−12.022	6.147	0.050*
		‡	206.358	−13.085	6.521	0.046*
Phosphate	maternal serum	†	3.309	0.417	0.160	0.010**
		‡	2.876	0.394	0.169	0.021*

SE – standard error; † simple linear regression analysis without adjusting any variables (the drinking water fluoride concentration was considered as an independent variable, and the serum ALP and phosphate levels were considered as dependent variables); ‡ multivariate linear regression analysis after adjusting for SES and maternal education; * statistically significant; ** highly statistically significant.

the fluoride concentration in maternal serum and cord blood also increased. According to the obtained results, the role of the placenta in blocking fluoride is very minimal. These results are consistent with previous studies by Ahmed et al.¹⁰ and Opydo-Szymaczek and Borysewicz-Lewicka.¹¹

Alkaline phosphatase has been identified as an early and vital indicator for the estimation of bone formation and bone turnover. There is no research pertaining to the effects of high and low/optimum fluoride concentrations

in drinking water on the bone marker ALP levels among pregnant women and in cord blood. In the present study, the mean ALP levels among pregnant women were higher than the cord blood ALP levels. This result is in accordance with a study by Verity et al.,¹² but contradictory to Yamaga et al.'s study.¹³

Some previous studies reported a weak inverse correlation between the maternal serum vitamin D3 and ALP levels.^{14–16} A few studies found an insignificant association between the vitamin D3 and ALP levels.^{17–19}

In the present study, lower ALP levels were noted in the high-fluoride group, which might be due to variations in the vitamin D3 levels. Those consuming high-fluoride drinking water had significantly lower ALP levels in maternal serum and cord blood than the low/optimum-fluoride group. Liu et al. showed that when the concentration of fluoride in drinking water was 0.58–1.59 mg/L, the ALP levels were higher, while fluoride concentrations of 1.60–3.37 mg/L were associated with lower ALP levels.²⁰ In the present study, the majority of samples that belonged to the high-fluoride group had fluoride concentrations of more than 1.5 mg/L and this group subjects exhibited lower ALP levels. These results are in accordance with the Liu et al's study.²⁰ Liu et al. showed that ALP activity was elevated in the low-fluoride group due to the direct stimulation of F, whereas in the high-fluoride group, fluoride could directly inhibit the enzyme activity or osteoblast activity.²⁰ Therefore, we consider that the ALP level may be one of the reference indicators for fluoride exposure.

In the present study, significant negative correlations were found between the fluoride content in drinking water, urine, maternal serum, and cord blood and the ALP levels. We found that the higher the fluoride level in maternal serum and cord blood were noted, the more decreased levels of ALP were observed. Further studies could be conducted to evaluate bone activity in pregnant women who consume high-fluoride drinking water.

Both Ca and P are essential inorganic elements for cell growth and proliferation. Phosphorus is important for DNA synthesis and for inducing mitogenesis. Due to higher bone turnover in the fetus, the values of cord blood Ca, P and bone metabolic markers are higher than the maternal serum ones.²¹ In this study, the low/optimum-fluoride group had higher phosphate values for cord blood than maternal serum. However, in the high-fluoride group, a reverse result was observed. The results also showed that the high-fluoride group had significantly higher phosphate values for maternal serum than the low/optimum-fluoride group. In cord blood, a reverse result was observed. This observation may be due to a lower secretion of parathyroid hormone (PTH) into maternal blood within the high-fluoride group as compared to the low/optimum-fluoride group.²² Previous studies showed that the secretion of PTH is directly linked to the P level. The uptake of phosphate from the intestine and bones into blood depends on the secretion of PTH. When a breakdown of bone occurs, more Ca than P is released into the bone. An increase in activated vitamin D mediates the absorption of both Ca and P in the intestine. The end result of the release of PTH is a slight decrease in the serum concentration of phosphate.²³

Limitations

There are limitations to this study that should be addressed. Firstly, fluoride was assessed only in drinking

water and not in other sources. Secondly, the study was conducted in a single center and had a small sample size. Thirdly, the cause-and-effect relationship could not be inferred with certainty, as this was a cross-sectional study.

Conclusions

The findings of this cross-sectional study demonstrated that a high fluoride content in drinking water was associated with the serum ALP and phosphate levels. We recommend multicenter studies with larger populations to establish the cause-and-effect relationship. Our data highlights that in high-fluoride areas, continuous screening of pregnant women should be performed to evaluate the ALP and phosphate levels. The government should take initiatives to create awareness regarding the effects of high fluoride levels in drinking water.

Ethics approval and consent to participate

The study was approved by the institutional ethics committee at the JSS Dental College and Hospital, Mysore, India (No. JSS/DC/Ethical/2014-15). All participants provided written informed consent.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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