ORIGINAL ARTICLE

The Effect of Dusts on Liver Enzymes and Kidney Parameters of Serum in Male Rats in Khuzestan, Iran

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KEYWORDS

Dusts; Kidney and liver enzymes; Metals toxicity; Khuzestan province; Iran

ABSTRACT: The present study aimed to investigate the effect of dusts on rat specimens in Southern Iran, Khuzestan, focusing on serum enzymes (Alpha-Amylase, Alkaline phosphatase, Alanine transaminase, Lactate dehydrogenase, and Aspartate transaminase) and kidney parameters (creatinine and urea). The study was conducted on 30 adult male rats that were divided into six groups, including one as the control group, and five as the experimental groups (Ahvaz, Ramhormoz, Andimeshk, Abadan, and Susangerd). Results showed that alanine aminotransferase increased in the groups of Ramhormoz, Andimeshk, Abadan, and Susangerd while Ahvaz showed a decrease. Alkaline phosphatase activity in all treated groups showed a significant increase compared to the control group. The activity of lactate dehydrogenase decreased in all samples, but only in Ahvaz and Susangerd groups it was significant compared to control group. Alpha-amylase activity was significantly increased in the groups of Ramhormoz, Andimeshk, Abadan, but decreased significantly in Ahvaz group, indicating a different value of harmful substances in dust particulates of these five cities. Serum urea level increased in all groups except Ahvaz, which was only significant in the group of Ramhormoz. Also, creatinine level decreased in all groups except Ahvaz. The results of this study showed that, the effect of fine dust was more evident in three cities of Ahvaz, Andimeshk and Ramhormoz due to the large amounts of total concentrations of heavy metals. Also, both the liver and the kidney were affected by both particulate matter, but it seems that the effect on the liver was more than the kidney.

INTRODUCTION

According to the recent studies, satellite images, and continuous samplings, the source of dust is from the coastal deserts of Africa [1]. Since a few years, dust particulates have attacked most of the southern and western parts of Iran and carried thousand tons of solid particulates from the deserts of Saudi Arabia, Yemen, Sudan, Iraq, Syria, Kuwait, and many other countries, causing trouble in the lives of people in southern, southwest provinces, and even in Tehran during stormy days. The dust phenomenon is usually accompanied by considerable masses of solid and suspended particulates that can be clearly detected by satellite imagery [2]. Dust storms are common in arid and semi-arid areas, which create a dust cloud up to 3000 meters. The density of this dust cloud is so high (almost 4,000 tons of dust per cubic km) that can completely block the vision [3]. Due to the

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The geographical position of Khuzestan, dust storms are more likely to blow to this province compared with other western regions [4]. In 2011, the World Health Organization announced Ahvaz as a city with the highest level of suspended particulate matter (PM) among 1110 cities in the world with a mean concentration of 372 μg.m⁻¹ [3]. WHO has placed suspended particulate matter in the first group of carcinogens. Due to their penetration into lung and bloodstream, the particulates cause permanent mutation in DNA, heart attacks, and premature death. In 2013, a study conducted in nine European countries showed that there is no immunity barrier against the penetration of these particulates into the lungs, and an increase in the concentration of suspended particulates with an aerodynamic diameter of 10 micrometers (PM₁₀) and 2.5 micrometers (PM₂.₅) per 10 micrograms will increase the lung cancer up to 22% and 36%, respectively [5]. Studies on suspended particulates in Ahvaz storms showed that these particulates have different size in terms of aerodynamic diameter, among which particulates with a diameter of 10 μm (PM₁₀) and 2.5 μm (PM₂.₅) are more permeable than other particulates and pass through typical breathing filters. These fine particulates have a large contact area and powerful absorption [6].

Particulates penetration into the body is dependent on their size, shape, and chemical composition. They can transfer a wide range of ionic, metal and organic compounds such as cations and anions, heavy metals such as lead, mercury, arsenic, and cadmium [6] as well as gaseous pollutants such as nitrogen dioxide, dioxygen, ozone, and carbon monoxide[3]. Organic toxic compounds are the result of incomplete combustion of fuels such as gasoline, diesel, coal and other chemical compounds. Toxic compounds along with the permeable particulates can penetrate the body through the respiratory tract, the digestive tract, and even through skin and cause various diseases [7].

Studies have shown that the concentration of fungi increases during dusty days. There is also a direct correlation between the increase of fungi in the dust and cardiovascular, respiratory, asthmatic and infectious diseases [8]. PM have a significant relationship with the concentration of bacteria in the air [8]. Ozone can oxidize lipids and proteins existing in the cell membrane [9]. Inflammation caused by PM₂.₅ leads to an increase in the number of neutrophils, eosinophils, T-cells and mastocytes [6]. The reaction of these particulates in pulmonary endothelial cells or erythrocytes may cause changes in red blood cells adhesion, indicating the effect of viral particulates on cardiovascular diseases [10]. An increase in the concentration of nitrogen dioxide and sulfur is correlated with the prevalence of chronic pulmonary disease [3]. The dust causes a disturbance in mitochondrial activity and depending on the time and concentration, increases H₂O₂ [11].

Organic aromatic compounds and quinones can cause mitochondrial damage and produce reactive oxygen species (ROS) [6]. The soluble metals in inhaled PM cause oxidative stress in the airway epithelial cells. A study by Becker et al., 2013 showed that ROS stimulates interleukin-8 production in human bronchial epithelial cells (HBE) and interleukin-6 in alveolar macrophages. Pulmonary injury is the result of 8-hydroxyguanosine induction as an indicator of oxidative stress in the lung epithelial cells and activated macrophages [6]. Experimental results of animal tests have shown that exposure to PM₂.₅ and very fine particulates can increase lipid peroxidation in liver and protein unfolding [12]. Antioxidants such as glutathione reduce inflammation of epithelial cells and tissue damage by ROS [13]. Exposure to PM increases the biomarkers of oxidative stress in the blood [14]. In addition to pulmonary diseases, dust may have health effects on other organs. Several studies have recently been conducted to show the effect of dust on cardiovascular diseases. The transfer of very fine particulates into the bloodstream can damage the heart. After sedimentation in veins, endothelial causes oxidative stress and inflammation, resulting in the accumulation of atherosclerosis plaques and thrombosis formation. Evidences suggest that exposure to suspended particulates results in the production of ROS, increasing the level of anti-inflammatory cytokines such as IL-8, IL-6, CRP and IL-1β. PMs may cause excessive ROS production, leading to defect in nitric oxide and increased coronary arteritis in laboratory studies. Also, exposure to
the particulates increases the plasma concentration of endothelin-1 (ET-1) [15].

In general, metal ions toxicity in the mammalian system is due to their chemical reactions with structural proteins, enzymes and membrane systems. The target of toxic metals is usually the ones with the highest concentration of metal. Depending on the types of heavy metals, their effects are different [16]. Heavy metals cause toxicity in living cells, leading to oxidative stress in them. Another biomarker of oxidative stress is lipid peroxidation. When free radicals accumulate electrons from the lipid molecules in the cell membrane, they cause peroxidation of the lipids. At high concentrations, ROS is likely to cause structural damage to cells, proteins, nucleic acids, and lipid membranes, resulting in a stress level at the cell surface.

Heavy metal ions quickly bond to albumin. Metals are easily bonded to the sulphydryl group at the terminal end of the cysteine and the histidine amino acid. Also, heavy metals are a potent inducer of metallothionein and glutathione in renal and hepatic tissues. Metallothioneins are low molecular weight cysteine-rich proteins [17]. Therefore, the present paper tends to investigate the effects of dusts on organisms. According to our findings, no study has been performed on biochemical indicators in mice under the influence of fine dust as animal models. In this study, for the first time, a comparative study of the fine dust effects on the activity of liver enzymes and serological factors related to the kidneys is performed among the cities of Khuzestan province.

MATERIALS AND METHODS

Dust analysis

The samples were sent to the Binaloud Laboratory (Tehran) to analyze the chemical compounds using the XRF method. Selected samples were studied by non-destructive analysis method of X Ray Fluorescence (XRF, Philips-1480 model).

Treatment of Animals and Experimental Design

While the peak arrival of dust in the province, the samples were collected from the different places such as homes, schools and mosques in five cities including, Ahvaz, Ramhormoz, Abadan, Andimeshk and Susangerd. This study was conducted on 30 Wistar rats weighing 180 to 250 grams. After transferring the animals, they were kept for one week in the new environment to prevent the effects of the potential stress of the new location and the possible change in their physiological condition. During the study, the animals were kept in proper condition, 12 hours of light and 12 hours of darkness, appropriate humidity and temperature of 23 ± 2 degrees centigrade. In this period, they were fed by urban water supply. The rats were kept in cages made of polycarbonate. During the study, all ethical considerations and working protocols on laboratory animals were considered.

The animals were randomly divided into six equal groups in separate cages (five in each cage) and examined in two general groups as follows:

- The control group that did not received any treatment, and the animals were fed only by urban drinking water and barley and sample groups which included all treated animals that weekly received 20 mg. Kg⁻¹ dust dissolved in 5 ml saline for two months.

Blood samples collection

In this study, the animals were kept for two months at an isolated place to examine the effect of dust. Their water and food were checked every day and cages were cleaned and then covered with sawdust. We check their food and water on these days. After two months and 12-hour hunger, all animals were anesthetized with diethyl ether and the samples were collected from their left ventricle. After 15 minutes, blood samples were centrifuged at 3000 rpm for 10 minutes. The serum samples were kept at a temperature of -20 degrees centigrade for until tests were performed.

Measurement of Serum Biochemical Parameters

The serum protein content was determined according to the Bradford (1976) method [18]. The absorption of this test is measured at 595 nm (140µl Distilled water, 40 µl Bradford solution and 20µl of sample). Then, serum enzymes
activities were measured based on the U/mg protein. The biochemical parameters of BUN (blood urea nitrogen) and creatinine were evaluated using laboratory kits (Pars Azmoon Co., Iran) according to manufacturer’s instructions. In this stage, urea and creatinine levels were measured by the enzymatic method of urease using a spectrophotometer (Biowave II spectrometer, biochrom Ltd, England) and the intensity of the color is proportional to the amount of urea in the sample at 600 nm and 490 nm for urea and creatinine respectively.

**Determination of Transaminase and Lactate Dehydrogenase Activity**

We determined the activities of the enzymes alanine transaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH) in serum samples using a commercial kit (Pars Azmoon, Iran) according to manufacturer’s instructions. Absorbance was measured at 340 nm using a spectrophotometer (Biowave II spectrometer, biochrom Ltd, England) based on an equal amount of serum.

**Determination of Alkaline Phosphatase Activity**

Alkaline phosphatase (ALP) activity was determined using a spectrophotometer (Biowave II spectrometer, biochrom Ltd, England). The enzymatic activity was carried out in serum samples based on an equal amount of serum using p-nitrophenyl phosphate (pNPP) as the substrate according to the kit’s instructions (Pars Azmoon, Iran). The instrument absorbance was adjusted to 405 nm and the sample measurement was carried out in the presence of a blank.

**Determination of alpha-Amylase Activity**

Amylase activity in the serum samples (pancreatic and salivary) was determined by using a diagnostic kit (Amylase kit, Pars Azmoon Co., Iran). The substrate was ethylidene-p-nitropheryl maltoheptaoside (EPS-G7). Absorbance, which is directly related to alpha-amylase activity, was measured at 405 nm and 37 degrees centigrade (Standard temperature) using an auto analyzer (BioTek Instruments, Inc. USA). Before application, the auto analyzer calibrated with the control sera N and P (TrueLab N® and TrueLab P®, respectively; Pars Azmoon Co., Iran) and a calibrator solution (TrueCal U®, Pars Azmoon Co., Iran). After calibration, the auto analyzer mixes 20 μl of serum sample with 1000 μl of substrate solution, automatically, and calculates the enzyme activity (IU/L) after a reaction delay of 1 minute 36 seconds. Finally, the specific alpha-amylase activity calculated as U/mg protein [19].

**Statistical analysis**

To ensure the normal distribution of the data, Kolmogorov-Smirnov test was performed on the test results. The assays were replicated three times. Also, independent t-test and ANOVA test were performed to compare the data and identify the difference between the groups. In all these comparisons, P <0.05 was considered as the significant difference and the results were represented as the mean SD.

**RESULTS**

**Dust element**

According to Table 1, which is based on XRF analysis, the distribution of heavy elements in dust samples varies in different cities. So that, elements such as Zn and Pb have the highest value in Ahvaz and Susangerd dust samples compared to others, while Al and Cr are more abundant in Abadan sample.
Table 1. Chemical analytical data of dust samples (concentrations of elements are in ppm)

<table>
<thead>
<tr>
<th></th>
<th>Pb</th>
<th>Se</th>
<th>Cr</th>
<th>As</th>
<th>Co</th>
<th>Al</th>
<th>Zn</th>
<th>Ba</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahvaz</td>
<td>123</td>
<td>363</td>
<td>84</td>
<td>4</td>
<td>4</td>
<td>5.42</td>
<td>1080</td>
<td>211</td>
</tr>
<tr>
<td>Andimeshk</td>
<td>36</td>
<td>1017</td>
<td>96</td>
<td>2</td>
<td>2</td>
<td>5.75</td>
<td>174</td>
<td>342</td>
</tr>
<tr>
<td>Ramhormoz</td>
<td>47</td>
<td>690</td>
<td>69</td>
<td>2</td>
<td>5</td>
<td>3.86</td>
<td>510</td>
<td>411</td>
</tr>
<tr>
<td>Abadan</td>
<td>36</td>
<td>578</td>
<td>136</td>
<td>4</td>
<td>8</td>
<td>6.87</td>
<td>251</td>
<td>175</td>
</tr>
<tr>
<td>Susangerd</td>
<td>82</td>
<td>608</td>
<td>117</td>
<td>3</td>
<td>3</td>
<td>6.71</td>
<td>412</td>
<td>102</td>
</tr>
</tbody>
</table>

Alkaline phosphatase

Alkaline phosphatase change was presented in Figure 1. In this regard, alkaline phosphatase activity in all treated groups showed a significant increase compared to the control group (p < 0.05). It was also indicated a significant increase in ALP activity in the Ramhormoz group as much as two times more than the control group.

![Figure 1](image1.png)

**Figure 1.** Alkaline phosphatase activity in control and sample groups. * shows that the difference is significant with control at p < 0.05.

Aminotransferases

Alanine aminotransferase variations are presented in Figure 2a. Accordingly, the activity of ALT increased in Ramhormoz and Andimeshk groups as compared with control. But a significant increase (P < 0.05) was observed only with Ramhormoz and Andimeshk groups.

Aspartate aminotransferase activity in Ramhormoz group decreased significantly (P < 0.05) compared to control group (Figure 2b). There were no significant changes in the enzyme activity of other groups as compared with control.
Figure 2. Alanine aminotransferase (a) and aspartate aminotransferase activities in control and samples groups. * shows that the difference is significant with control at p < 0.05.

Lactate dehydrogenase

According to Figure 3, only in Ahvaz and Susangerd groups the changes in its activity was significant compared to control group (p < 0.05). In addition, the enzyme activity in Ramhormoz, Andimeshk and Abadan groups did not show any significant change (p > 0.05).

Alpha-amylase

According to Figure 4, the activity of alpha-amylase increased significantly (p < 0.05) compared to the control in all samples except Ahvaz. However, this increase was much more pronounced in the two groups of Ramhormoz and Andimeshk. Meanwhile, the Ahwaz sample showed a significant decrease of alpha-amylase activity as compared to the control group (P < 0.05).
In Figure 5 urea concentration in Andimeshk, Abadan, Ahvaz and, Susangerd Groups compared to the control group is shown. Urea concentration increase in Ramhormoz sample comparing to the control group was only significant (p < 0.05).

According to Figure 6, the creatinine levels did not change significantly in other cities except Ahvaz, which showed a significant increase compared to the control group (P < 0.05).
DISCUSSION
Air pollution is a severe problem in the residential and industrial areas worldwide. Ahvaz, the center of Khuzestan province, is the second-largest city in Iran after Tehran. It is a city with many contaminated areas due to its abundant oil and gas resources, petrochemicals, large metal and nonmetallic industries, cellulosic and electricity industries, as well as humid climate. In addition, dust is another air pollutant in Ahvaz [8].

The metals toxicity has been well documented. One of the most essential mechanisms of heavy metal toxicity is oxidative stress. Studies have shown that the heavy metals can interact with nuclear and DNA proteins, causing oxidative macromolecules to activate biologically. Also, metals such as iron, copper, cadmium, mercury, nickel, lead and arsenic are capable of producing reactive radicals that cause cell damage, such as reduced enzymatic activity, damage to the lipid bilayer membrane and DNA. They include a wide range of oxygen, nitrogen, carbon and sulfur radicals that produce superoxide, hydrogen peroxide, lipid peroxide, amino acids, peptides, and proteins radicals. Compounded with heavy metals, these components cause diseases such as liver toxicity and neurological disorders [20]. In this paper, the effect of dusts on liver enzymes and kidney parameters of serum in male rats in Khuzestan, Iran are discussed.

ALP is a hydrolyzing membrane enzyme that causes the phosphate group to be transferred from a variety of molecules, including nucleotides, proteins, and alkaloids. The main function of the alkaline phosphatase is probably to facilitate the transfer of various cell membrane metabolites associated with the transport of fat and ossification. This enzyme is active in most tissues of the body, but the highest level of activity is found in the intestines, liver, bone, spleen, and kidneys. Studies have shown that the activity of alkaline phosphatase is a marker of liver and bone diseases. It has also noticeable changes in many diseases including autoimmune diseases, infectious diseases, liver, biliary, bone, inflammatory conditions, and anemia [21, 22]. The results of the present study indicated that this enzyme significantly increased in five treatment groups. The results of a study by Taten et al., 2015 showed the increase of alkaline phosphatase activity [23], which, though contradicted the results of Singh [24], were consistent with the results of the present study. As mentioned, ALP may be an indicator of liver and bone damage that is associated with an increase in osteoblast. Although toxicity due to the heavy metals can increase the concentration of ALP in the bone, heavy and toxic metals can reduce the synthesis of protein and type I collagen fibers [25]. The results of dust analysis showed that the groups of Ramhormoz, Ahvaz, and Susangerd have the highest value of barium, lead, and Chromium respectively, while the value of Arsenic in the five groups did not show a significant change. Interaction of heavy metals such as lead (Pb), Zinc (Zn) and Aluminum (Al) with active site of ALP increase the serum ALP activity. However, the increase in enzyme activity can also be attributed to bile ducts injury (due to the action of heavy elements) [26]. Hepatic cells, as complex metabolic cells, contain high levels of enzymes. Aminotransferases are critical hepatic enzymes that play an essential role in the metabolism of amino acids and the transfer of the amine groups between

Figure 6. mean value of creatinine in control and samples groups. * shows that the difference is significant with control at p < 0.05.
an amino acid and α-ketoacid. Among them, two enzymes of ALT and AST are important in terms of activity. AST is naturally found in a variety of tissues such as liver, heart, muscle, kidney, pancreas, brain and lungs. This enzyme enters the blood at the time of damage of these tissues, in particular, damage to the heart tissue. In contrast to AST, the major part of ALT is found naturally in the liver and kidney. The presence of ALT and AST enzymes in the serum and their increased activity mainly results from the hepatic parenchymal cells damage. Therefore, they are used to detect liver diseases [27, 28].

The results of this study showed that ALT increased in groups of Ramhormoz, Andimeshk and Susangerd, and AST in groups Abadan, Susangerdi and Andimeshk. This increase of enzyme depends on the dose of dust particulates [29]. On the other hand, in groups of Ahvaz and Abadan, ALT decreased, and in the Ramhormoz, there was a decreasing trend in AST. Other studies reported the same trends. Consequently, liver function and the degenerative changes probably terminated to the observed changes in ALT and AST [30, 31]. This is also due to the heterogeneity of the compounds in the suspended particulate matters. In 60% of cases, increase of AST is due to liver toxicity. According to the table 1, metals such as Cr and Pb of dust samples in the group of Ramhormoz were lower than other groups and probably caused the decrease of AST in this group. Also, the increase of these enzymes is perhaps due to increased cell membrane permeability, cell membrane destruction and hepatocytes by heavy metals in dusts. For example, Aluminum toxicity in the dust is associated with various biochemical disorders that contribute to the release of intracellular elements in the circulation of the blood. Therefore, after weakening membrane integrity or interfering with the function of normal metabolism, it can lead to a change in the activity of these enzymes [32].

Lactate dehydrogenase is an oxidoreductase and intracellular enzyme that catalyzes the reciprocal reaction of pyruvate with lactate. LDH is found to be abundant in the cytoplasm of all body tissues, including the heart, liver, kidney, red blood cells and skeletal muscle. It is usually increased gradually from 24 to 48 hours after stimulation. The enzyme has five iso-enzymes, each with different concentrations in various tissues. This enzyme is a metal-zinc dependent, which is synthesized in the body and strongly influenced by the value of zinc [33]. The results of the present study showed that LDH activity was significantly decreased in the treatment groups except Ramhormoz, Andimeshk and Abadan. This variation was consistent with the results of other studies [34, 35]. The increase or decrease of LDH activity in serum is probably due to hepatocyte damage. LDH enzyme also plays an essential role in the metabolism of carbohydrates. When the treated animal was exposed to the thrombocyte, the activity of the LDH would inhibited and eventually lead to the accumulation of lactic acid, the final product of anaerobic glycolysis. As mentioned, heavy metals and elements in the dusts cause oxidative stress, and thus inhibit cellular oxidation and LDH activity. This indicates that the anaerobic activity of the cell is inhibited due to air pollution, and may lead to weaken the biochemical functions of the body [36]. The activity of this enzyme was significantly decreased in Ahvaz and Susangerd groups. Total toxic metals such as arsenic, chromium and zinc in these two groups had high value.

Alpha-amylase is a glycoside hydrolase family with a molecular weight of 50 kDa. This group is endoglycosidase that break down carbohydrate chains such as starch or glycogen. Two main types of alpha-amylase in mammals include salivary and pancreatic, which are mostly similar in amino acid sequences [37]. Based on the present results, alpha amylase activity was significantly increased in the groups of Ramhormoz, Andimeshk and Abadan. However, there was a significant decrease in Ahvaz group, indicating disimilarity between harmful matters in the dust of these five cities. The results were inconsistent with the results of study by[38]. Heavy metals (pb, cd and se) in the dust increase the activity of alpha-amylase, which can be the result of increase in concentration of the enzyme in the blood or decrease in its excretion. The pancreas or inflammation damages cause hyper amylases, which its reduction can also be due to kidney failure [39]. The kidney is the main source of filtration and one of the detoxification sites in the body. Blood urea nitrogen and
creatinine are of the most common factors for the evaluation of renal function, which are strictly related to glomerular filtration [40]. Ammonia in the body results from protein metabolism, and converted to urea in the liver to the detoxification purposes. Blood urea concentration can be increased for several reasons such as increased protein degradation, gastrointestinal bleeding, chronic and acute conditions, shock, muscle atrophy and some chronic hepatic and renal diseases [41]. In the present study, the urea level of serum taken from all samples was however increased but it was significant in ramhormoz sample (Figure 5). Creatinine is the product of creatin metabolism in the muscles that is excreted through the kidney. Creatinine values in blood flow is controlled by kidneys activity, so measuring the creatinine can be indicative of the functioning of the kidneys. In this regard, when their function is reduced, the value of creatinine increases in the blood [42]. In this study, the level of creatinine decreased in all groups, except for Ahvaz (Figure 6). The results were consistent with the results of study by Shrivastava (2011). Increase of urea concentration in the serum of Ramhormoz group may be due to increased phosphate in dust samples. Therefore, it seems metals can have different effects on biochemical parameters; for example, chromium reduces concentration of serum urea and leads increase the creatinine [43].

CONCLUSIONS

The present study aimed to determine the effect of dust particles on liver and kidney serum enzymes. The results indicated that the toxicity of heavy elements in dust was different in the studied locations which might be attributed to settling of the particulates. In general, it seems that the high values of total heavy metal concentrations in Ahvaz, Andimeshik and Ramhormoz groups have led to more changes in the analytes which were measured in these groups. Comparing the results of this study, both liver and kidney were affected by particulate matter, but it seems that the effect on the liver was more than the kidney. Finally, since the effect of environmental pollutions on living organisms usually does not manifest itself in the short-term, it appears that the lack of significant changes in some groups under treatment was due to the biological tissues repair, therefore, the authors strongly recommend that the long-term effect of fine particles on the biochemical parameters of the liver and kidneys be studied.

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