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Original Citation:

Analysis of Particulate Pollution on Foodstuff and Other Items by Environmental Scanning Electron Microscopy / C. GIORDANO; U. BARDI; D. GARBINI; M. SUMAN. - In: MICROSCOPY RESEARCH AND TECHNIQUE. - ISSN 1097-0029. - ELETTRONICO. - 74:(2011), pp. 931-935. [10.1002/jemt.20978]

Availability:

The webpage <https://hdl.handle.net/2158/776884> of the repository was last updated on

Published version:

DOI: 10.1002/jemt.20978

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Analysis of Particulate Pollution on Foodstuff and Other Items by Environmental Scanning Electron Microscopy

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KEY WORDS ESEM; EDX; inorganic particules; everyday food items; organic food; human health

ABSTRACT Combustion processes commonly create fine and ultrafine particles whose effects are often harmful to human health. The present study is aimed at providing more data in this field by testing the capability of environmental electron scanning microscopy of detecting and analyzing such particles. For this purpose, we examined a range of samples taken from everyday food items collected in Tuscany. The results showed that, within the examined samples, inorganic particles can be observed in the nano- and micrometer range. These particles are attributable mostly to natural processes and, in part, to food processing. Little evidence is found for particles whose origin could be attributed to industrial combustion processes, such as waste incineration. *Microsc. Res. Tech.* 74:931–935, 2011. © 2011 Wiley-Liss, Inc.

INTRODUCTION

In some recent studies (Ballestri et al., 2001; Bregoli et al., 2009; Gatti, 2004; Gatti and Montanari, 2008; Gatti and Rivasi, 2002), the combination of environmental electron microscopy (ESEM) and energy-dispersive X-ray spectroscopy (EDAX) has been used to study the presence of inorganic fine and ultrafine particles in the human body and in a variety of biological substrates, including foodstuff. The detected particles have often been attributed to pollution emitted by industrial combustion processes. These particles may diffuse inside the human body by direct inhalation (Andujar et al., 2009) but may also be first absorbed in food and then diffused into the human body through the digestive process (Gatti and Montanari, 2008). The process of inflammation associated to ultrafine particles is known to be a possible cause of tumors (see, e.g., Gatti, 2004; Gatti and Rivasi, 2002), and evidence is accumulating on the harmful effects of nano and microparticles in human tissue (Andujar et al., 2009; D'Anna, 2009; Rejnders, 2006; Sharma and Sharma, 2007). As a consequence of these studies, the term “nanopathology” has been coined (Gatti and Montanari, 2008) in order to describe the harmful effects of fine particulate inside the human body.

In the present work, we add some elements to this field of studies by presenting the results of a survey carried out by ESEM and EDAX on a variety of biological samples, mainly, foodstuff. Our main objective was to verify whether the results reported in Gatti and Montanari (2008) could be replicated on a different set of samples and, in particular, to verify whether we could detect inorganic particles emitted as the result of industrial combustion processes. Such particles would be recognizable by their shape and composition (Franck and Herbarth, 2002). Particles that are the

result of high-temperature combustion processes should show spherical shapes, in contrast with particles, which are the result of mechanical wear—as in natural and industrial processes—which are expected to be irregular in shape. The composition, also, is an indicator of industrial origin as we expect to detect the presence of elements (e.g., heavy metals), which are uncommon in nature.

We observed the presence of inorganic nano- and microparticles in foodstuff collected mainly in Tuscany, Italy. In most cases, the observed particles can be identified as ordinary dust and, in some cases, to mechanical processing of the materials examined. No clear evidence was found of particles that could be attributed to airborne particles resulting from combustion processes. Obviously, the available sets of data are insufficient to provide statistically significant results, but we believe that presenting these data is a first step toward obtaining a wider knowledge of the diffusion of inorganic particulate in Europe and in the world.

MATERIALS AND METHODS

A total of 62 samples were examined. The samples were obtained from food bought at local markets or supermarkets. Of these, 26 were labeled as “organic” food. A complete list of the examined samples is reported in Table 1

All the measurements reported here were performed in an ESEM FEI QUANTA 200. Samples were intro-

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Received 29 July 2010; accepted in revised form 17 November 2010

DOI 10.1002/jemt.20978

Published online 11 April 2011 in Wiley Online Library (wileyonlinelibrary.com).

TABLE 1. List of the examined samples

| Type of sample | Number examined | Origin |
|---------------------------------------------------------------------------------------------------------|-----------------|-----------------------------------------------------------------------|
| Fruits | 10 | Florence area |
| Vegetables | 11 | Florence area |
| Meat (minced and homogenized) | 5 | Commercial processed food from Italy |
| Fish (tuna) | 1 | Commercial canned tuna – from Atlantic Ocean |
| Cheese | 3 | Commercial processed cheese from Italy |
| Dry mushroom | 1 | Commercial packaged mushrooms from Italy |
| Packed cereals products (croissant, snacks, pasta, rice, bread, biscuits, crackers, rusk, and croutons) | 19 | Commercially packaged products, mainly from Italy |
| Unpackaged food (eggs, flour, sugar, salt, and cocoa) | 6 | Commercial products, mainly from Italy. Cocoa from Dominican Republic |
| Assorted plants (plane tree, lichen, rosemary, and ear of wheat) | 5 | Samples collected in Florence and outskirts |
| Bamboo cane roof | 1 | Sample collected in Florence |

duced in the measuring chamber with no pretreatments or minimal ones as specified here. The outer surface of fruits, vegetables, and plants was examined without peeling. For banana and onion also, the interior part was examined. In the case of potatoes, pumpkin, zucchini, and plums, a second test was performed after washing in water. Potato and pumpkin were peeled and then washed a second time to simulate the effect of food preparation on the adsorbed particle on the surface. Biscuits, rusk, and croutons were crumbled before analysis.

An area of $\sim 4 \text{ cm}^2$ was analyzed for each sample. Inorganic contaminant particles were clearly visible as lighter spots in electron backscattering mode.

The chemical composition of the particles was determined by EDAX. The area analyzed by EDAX in our experimental condition corresponds to a spot of $\sim 50 \text{ nm}$ of diameter. However, the volume examined can be estimated as a few cubic micrometers.

RESULTS

The results obtained in this survey are reported in Tables 2 and 3. All the examined samples showed the presence of inorganic particles in the range from nanometers to micrometers. These samples could be easily detected in backscattering mode in ESEM as bright spots over a dark background. The brightness of these foreign bodies was due to the higher atomic number of the elements in the particles, in comparison with the substrate, which was mainly composed of carbon and oxygen. A large variety of sizes was observed, but we decided to examine mainly particles of size smaller than $\sim 100 \mu\text{m}$ as larger sizes can be considered as macroscopic particles, unlikely to penetrate the human body. The analysis by EDAX provided in all cases a good signal/noise ratio that permitted us to identify the composition of the particles.

Representative examples of the images obtained are shown in the Figures 1–6.

TABLE 2. Atomic elements detected on food and plant samples by EDAX analysis

| Element | Prevalence | Sample |
|---------|------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Al | 45/62 | Packed alimentary products, organic packed alimentary product, plants, meat, raw material for food, organic vegetable, vegetable, fruits, cheese, mushroom, organic raw material for food, organic fruits, and bread |
| Si | 44/62 | Packed alimentary products, organic packed alimentary product, plants, meat, raw material for food, organic vegetable, vegetable, fruits, cheese, mushroom, organic raw material for food, organic fruits, and bread |
| Fe | 44/62 | Packed alimentary products, organic packed alimentary product, plants, meat, raw material for food, organic vegetable, vegetable, fruits, mushroom, organic raw material for food, organic fruits, and bread |
| Ni | 2/62 | Packed alimentary products |
| Cu | 8/62 | Packed alimentary products, organic packed alimentary product, plants, meat, and bread |
| Zn | 9/62 | Packed alimentary products, organic packed alimentary product, bread, meat, and organic vegetable |
| Ti | 12/62 | Packed alimentary products, organic packed alimentary product, plants, vegetable, organic fruits, and organic vegetable |
| Pb | 1/62 | Bread |
| Sr | 1/62 | Organic raw material for food |
| Cr | 1/62 | Packed alimentary products |

DISCUSSION

In all cases, the observed particles had irregular shapes, and nowhere we observed regular spherical shapes that could be the result of exposure at high-temperature conditions (Franck and Herbarth, 2002). The EDAX analysis showed that, by far, the most commonly observed metals were aluminum, silicon, and iron. These are natural components of ordinary dust, such as clay mineral deposits based on aluminosilicates. Such particles cannot be considered as contaminants due to industrial activities. Titanium was not observed in the form of pure oxide, and so it cannot be attributed to a component of common pigments. Rather, it is a naturally occurring element in granite, and this is most likely its origin.

Of the remaining elements, copper and zinc were observed in few cases on bread, and other products were derived from wheat. It is very likely that these particles of irregular shape are the result of mechanical wear of bronze parts of the machinery used in the food industry. Also, chromium, occasionally observed, may be attributed to the wear of stainless steel tools used in food processing. Strontium is also a component of some naturally occurring minerals in the form, for instance, of strontium carbonate (Strontianite). We also considered the hypothesis that the presence of strontium may derive from contamination from the electrodes of lead batteries for which strontium is reported to be a possible alloying element (Bagshaw, 1991). However, we rejected this hypothesis after a survey that indicated that batteries containing this element do not exist in the market at present (Fernandez, 2010; private communication).

TABLE 3. Atomic elements and size of particles found in food and plant samples

| Element | Range of size (μm) | Average (μm) | Modal score | 25th percentile | 50th percentile | 95th percentile | Standard deviation |
|---------|---------------------------------|---------------------------|-------------|-----------------|-----------------|-----------------|--------------------|
| Al | 0.5–100 | 18 | 10 | 4 | 10 | 70.5 | 22.6 |
| Si | 0.5–100 | 19.7 | 10 | 4.1 | 10 | 70 | 23.5 |
| Fe | 0.5–100 | 19.4 | 10 | 5 | 10 | 59.5 | 21.1 |
| Ni | 1–2.5 | 1.8 | | 1.5 | 2 | 2.45 | 0.6 |
| Cu | 2–50 | 12 | 10 | 4.7 | 9 | 43 | 13.4 |
| Zn | 2.5–40 | 11.1 | 5 | 6.5 | 9 | 26 | 8.7 |
| Ti | 2–80 | 26.9 | 15 | 9 | 20 | 5.3 | 21.7 |
| Pb | 12 | 12 | | | | | |
| Sr | 10 | 10 | | | | | |
| Cr | 2 | 2 | | | | | |

The average size of the particles is calculated over the total number of detected particles.

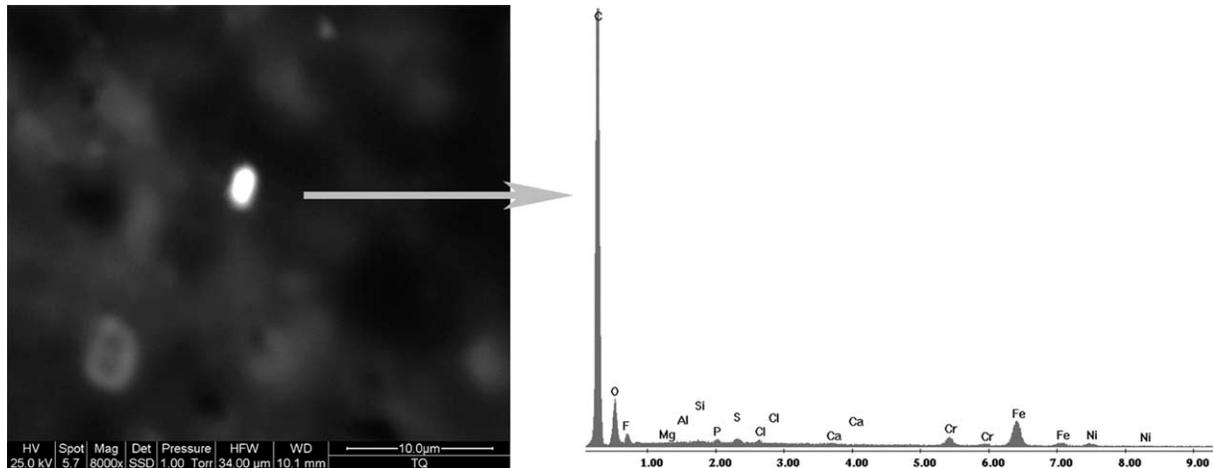


Fig. 1. Particle observed in packed croutons. The size is of about $1 \mu\text{m}$, and the composition is mainly iron oxide with a fraction of chromium and nickel. It is a composition typical of commercial stainless steel, and this particle is probably the result of mechanical processing of the food.

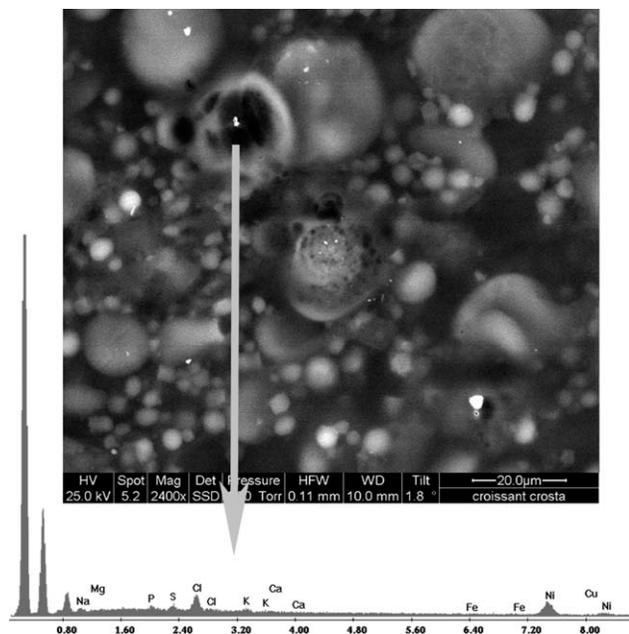


Fig. 2. Particle found in packed croissant and the relative spectrum. This particle is in the nanometer range, and it contains sodium, chlorine, and nickel. Sodium and chlorine exist most likely in the form of sodium chloride, while nickel is probably deriving from a pure nickel anticorrosion coating. The presence of sodium chloride and the smallness of the particle indicates a likely airborne origin. This is one of the few particles observed that can be attributed to such an origin.

In the end, the only contamination that might be attributed to industrial pollution is that of lead, observed in just two samples. However, because the particles observed are rather large ($\sim 10 \mu\text{m}$), it is unlikely that they could have been transported in the atmosphere as dust. In principle, these particles might be the result of direct contamination of the environment from improperly discarded lead batteries.

Our results can be directly compared to two other studies previously reported in the literature. One of these studies (Brighigna et al., 2002) reported the presence of PM 10 particles detected by SEM in tissue from Tillandsias plants collected in Florence. The composition of the particles was not reported, and it appears that these particles were mainly carbonaceous, that is, soot. The other study (Gatti, 2004; Gatti and Montanari, 2008) used ESEM on a variety of foodstuff samples, observing several types of inorganic particles, which were interpreted, at least in part, as the result of contamination deriving from airborne particles in turn created by industrial processes.

CONCLUSION

Micro and nanoparticles introduced in the body via respiratory system can cause inflammatory stresses; on the other hand, toxicological evaluation on the effects from ingestion is meager, and toxicity evidence still do not exist for the long term. We are facing a considerable challenge in trying to understand the effect

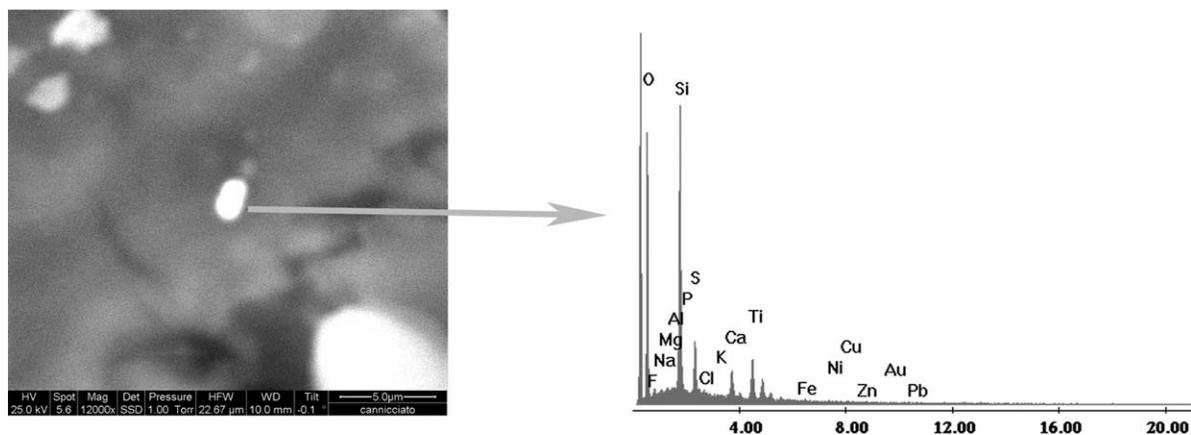


Fig. 3. Particle found in bamboo cane roof. The size is of about 2 μm . The elements observed, mainly silicon and titanium, are components of ordinary dust resulting from minerals commonly contained in the earth's crust.

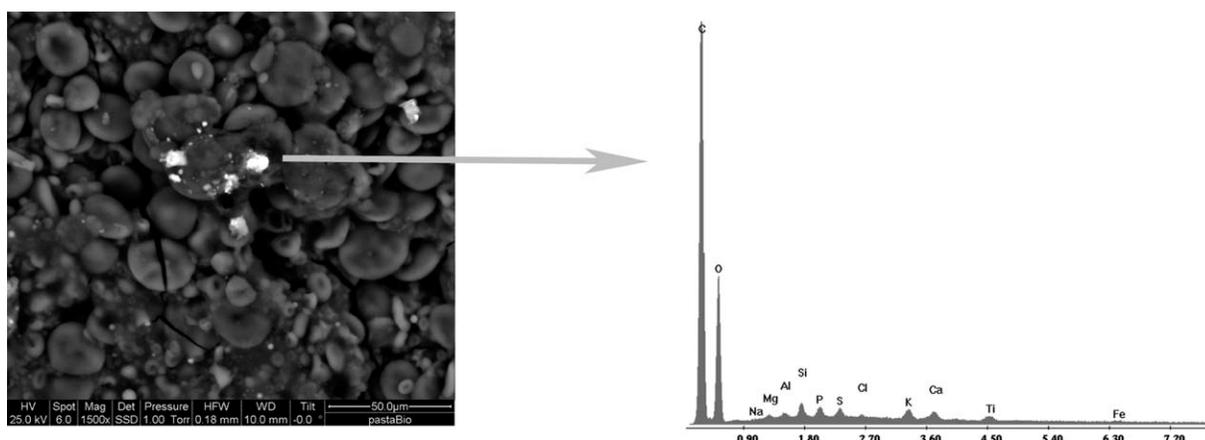


Fig. 4. Particle observed in a pasta sample labeled as "biological food." The size is of about 1 μm . The elements observed, silicon, titanium, phosphorous, and aluminum are components of ordinary dust resulting from organic matter.

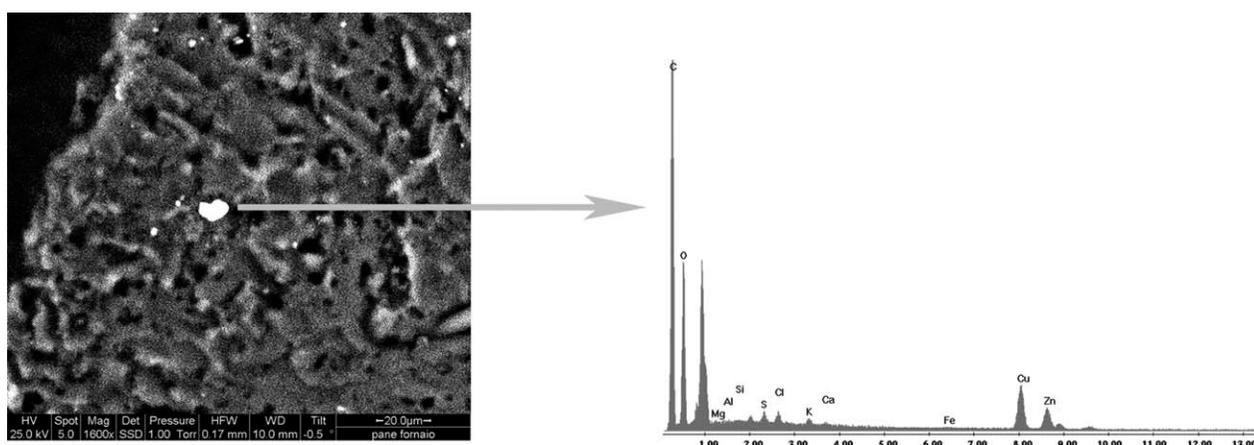


Fig. 5. Particle observed in a bread sample. The size is of about 4 μm . The elements observed, copper and zinc, are clearly derived from bronze tools used during processing.

of these particles on human health. The present work has examined the capability of electron microscopy (ESEM) to detect inorganic particles in foodstuff. We

find little or no evidence that inorganic particles, resulting from industrial processes, in particular, of high-temperature combustion processes are a common

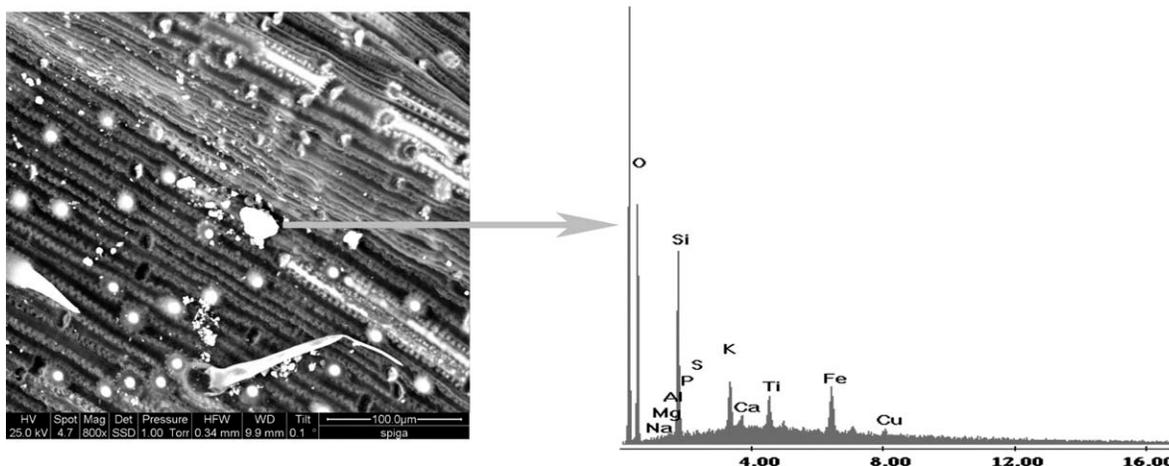


Fig. 6. Particle of 34 μm found on an ear of wheat. It contains mainly natural elements (silicon, iron, titanium, calcium, and potassium). Traces of copper of uncertain origin can be observed.

contaminant of foodstuff and other everyday items. Most or all of what was observed can be attributed to naturally occurring elements and, in minor amounts, to wear of machinery used in food processing. These results are therefore in apparent contrast to those reported in references (Gatti, 2004; Gatti and Montanari, 2008), where the inorganic particles observed by ESEM were interpreted mainly as the result of industrial combustion processes. The different results may be due to the different geographical areas examined by our work and by those in references (Gatti, 2004; Gatti and Montanari, 2008). This discrepancy highlights the limits of the ESEM analysis, which have to do with the fundamental problem of transforming data obtained on small areas of a limited number of samples into data that can tell us what is the distribution of the observed particles within a macroscopic geographic area. If ESEM data are not combined with statistically significant data, the results will always be open to different interpretations. Therefore, we believe that the significance of our work lies mainly in highlighting the strong points and the limitations of this kind of analysis. ESEM provides a characterization of small particles that no other technique can provide. Nevertheless, it should not be used as a substitute for epidemiological studies nor it can be used as sole instrumental approach for claiming hypothetical effects of industrial processes on human health. Still, ESEM may prove to be a valuable tool in this area if the database recorded can be enlarged by future studies, and if these outputs are combined with the complementary support of other

analytical techniques and dedicated sample treatment strategies.

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