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Nano particle Collection by Various Sampling Techniques for Worker Exposure Assessment

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Abstract

Introduction: This study was aimed to evaluate eleven sampling methods currently available for assessing nanoparticle exposure for workers; and study three devices designed to collect nanoparticles on transmission electron microscope grids.

Materials and Methods: Nanoparticle collection was evaluated by generating three aerosols in a controlled environment; and the particle deposition and size were analyzed using direct reading instrument, electron microscope and image analysis.

Findings: Particles less than 100 nm were collected and counted with a large portion in this range on sampled grids, regardless of the aerosol or sampler used. However, particle deposition differed slightly for different test aerosols may be affected by thermal resistance and electrical resistivity of particles.

Conclusion: Collecting nanoparticles directly onto grids is an effective technique. All of the direct-deposition methods offer distinct advantages due to the direct analysis post collection.

Introduction

Nanoparticles such as carbon-based particles and metal oxides have been found to induce inflammatory responses in human cells which could be associated with various potential health effects [1-14]. Carbon nanotubes and Titanium Dioxide (TiO₂) nanoparticles were reported to be potential human carcinogens [15,16]. Exposure to nanoparticles in humans usually occurred in an uncharacterized work environment and could cause irreversible adverse health effects. For example, worker exposure to nickel nanoparticles caused pulmonary and systemic toxicity and developed sensitization [17,18]. Adverse effects for workers exposed to nanometer-sized aerosols in general have recently been reported [19-26]. This evidence raises concern and emphasizes the importance of characterizing novel nanoparticles and their unknown adverse exposure effects. Various toxicology studies

have raised the possibility that particle number and size may be important exposure metrics, rather than the mass concentration typically evaluated for larger particles.

The particle metrics most commonly used by researchers in published workplace nanoparticle exposure studies are mass, number concentration and size distribution [27]. A variety of direct reading real time instruments (RTIs) are available to measure these metrics but are unable to distinguish between incidental nanoparticles and those coming from a process of interest [27]. Using gravimetric personal samplers to measure the mass concentration of nanoparticles is limited by the sensitivity needed to detect the typically small mass concentration of these particles [28]. Issues such as these contribute to the need for the microscopic analysis of nanoparticles. Transmission Electron Microscope (TEM) image analysis of nanoparticles has been conducted by taking images of

particles and using image analysis software to measure particle size, agglomeration, and elemental composition through Energy Dispersive X-Ray spectroscopy (EDX) [29-38]. TEM analysis allows for the selection of specific particles to be tested for compositional analysis, as compared to the bulk sample analysis that can be done with filter-based samples. Based on knowledge to date, issues in performing personal exposure assessments include difficulty in collecting nanoparticle samples from the breathing zone and the corresponding lack of a broadly applicable method for this purpose; thus, more research is needed on developing a method for collecting and analyzing nanoparticles in occupational environments [12].

New sampling techniques to assess personal exposure as an important initial step for exposure assessment have been developed in recent years, but this work is still in the developmental stage [39]. Workplace nanoparticle exposure assessments often utilize both area and personal sampling. Assessments were typically performed using RTIs to measure concentration and using samplers to collect nanoparticles for subsequent analysis [28,33,40]. Several new approaches to sample nanoparticles have become available, all of which utilize deposition mechanisms other than the impaction used in gravimetric sampling [41-45]. Due to the minimal effect of inertia and gravity on small particles, collecting nanoparticles

would theoretically be more effective using a thermal gradient, electrostatic field and diffusion-Brownian motion rather than inertial impaction [46]. The substrates used to collect nanoparticles include small filmed TEM grids and membrane filters (rather than the fibrous filters used in gravimetric sampling) allow direct microscopic particle analysis. The information available from this approach includes particle morphology, size, agglomeration status, and elemental composition, none of which is obtainable from gravimetric sampling.

Nanoparticle Sampling Approaches

Various sampling approaches have been developed to characterize nanoparticles, which can be categorized as gravimetric methods and non-gravimetric methods, and both have been employed for nanoparticle assessments. Sampling methods currently designated for assessing nanoparticle exposure in compliance for recommended exposure limits (RELs) in the US include National Institute for Occupational Safety and Health (NIOSH) Methods 7402, 5040 and 0600 [47-49]. In addition, several newly developed sampling devices are discussed in this study to understand the current status of sampling technology. Features of each method are summarized in Table 1, following with descriptions of each method or sampler evaluated in this paper.

	Substrate	Measurement	Sampling Period	Microscope Analysis	Particle Type	Operating Flow rate	Collection Mechanism	Particle Size Range	Collection Efficiency
7402	MCE filter	Particle count	Long, Short	TEM	Fiber	5L/min	Impaction, interception, diffusion	Inhalable	Not specified
5040	Quartz filter	Mass	Long	N/A	Carbonaceous	2.0-4.0 L/min	Impaction, interception, diffusion	Inhalable or respirable	Not specified
600	PVC filter	Mass	Long	N/A	TiO ₂	1.7L/min	Impaction, interception, diffusion	Respirable	Not specified
PGP	Filter	Mass	Long	N/A	Fiber, Non-fiber	2L/min	Impaction, interception, diffusion	Inhalable	Not specified
Nano-badge	Polycarbonate membrane filter	Mass	Long	SEM	Not specified	0.6 or 1 L/min	Impaction, interception, diffusion	Respirable with pre-separator	Not specified
Partector	Grid	Particle count	Long	TEM	Not specified	0.45 L/min	Impaction, diffusion	Nanoparticles	50 nm 3%*
PEN	Teflon membrane filter	Mass	Long	N/A	Not specified	2L/min	Impaction	Respirable	Rotating impaction: 200 nm 94%, 560 nm 85%, < 100 nm below 50%*

NRD	Teflon membrane filter, nylon mesh	Mass, Particle count	Long	SEM	Non-fiber	2.5L/min	Impaction, interception, diffusion	Respirable	Impaction: 15 nm 26%, 100 nm 8%, 550 nm 96%; <100 nm follow ICRP model*
TPS	Grid	Particle count	Long, Short	TEM	Not specified	0.005 L/min	Thermophoretic force	20-600 nm	600 nm 60%*
ESP	Grid	Particle count	Short	TEM	Not specified	0.055 L/min	Electrostatic field	30-400 nm	86% Max.
TDS	Polycarbonate membrane filter, grid	Mass, Particle count	Long, Short	TEM/SEM	Fiber, Non-fiber	0.3L/min	Impaction, interception, diffusion	Respirable	All entering sizes, close to 100%

Details regarding available size fractioned efficiency are described in Nanoparticle Sampling Approaches.

Inhalable: Inhalable size of particles is defined as particulate matter with a mean aerodynamic diameter of 100 microns (µm) or less and is practically defined as that size fraction of particulate which is able to enter the nose and mouth in the respiratory tract.

Respirable: Respirable size of particles is defined as particulate matter with a mean aerodynamic diameter of 4 microns (µm) or less and is practically defined as that size fraction of particulate which is able to reach the alveoli in the respiratory tract.

Nanoparticle: Particles with primary size in the length scale of approximately 1-100 nanometer (nm) range.

7402: US NIOSH 7402 method for fiber sampling and analysis

5040: US NIOSH 5040 method for diesel exhaust and elemental carbon sampling and analysis

0600: US NIOSH 0600 method for titanium dioxide ultrafine particle sampling and analysis

PGP: person engetragenes Probenahme system (in German)

PEN: personal nanoparticle sampler

NRD: nanoparticle respiratory deposition sampler

TPS: thermophoretic nanoparticle sampler

ESP: electrostatic precipitator

TDS: Tsai Diffusion Sampler

ICRP: International Commission on Radiological Protection

Table 1: Features of sampling methods and samplers for evaluating worker personal exposure to nanoparticles.

Filter based sampling to obtain the mass concentration or fiber count has been the accepted approach to quantify particulate exposure since the 1970s. A similar approach has been employed to quantify exposure to metal oxide nanoparticles, such as Titanium Dioxide (TiO₂), with filter-based particle collection to obtain mass concentration. Recently a modified version of method 7402 [38] from the NIOSH Manual of Analytical Methods (NMAM) has been used to collect and analyze carbon nanotubes by noting their shape, dimensions, and clustering. Progress has been made in creating a NMAM procedure [31] designed specifically for carbon nanotubes (CNT) and carbon nanofibers (CNF). It uses open-face, 25-mm, three-piece cassettes, for air sampling approximating the inhalable fraction [38, 50]. As specified in NMAM 7402, particles are collected on mixed cellulose ester (MCE) filters having a nominal pore size of 0.8 µm with an air flow of 5 L/min [31]. CNT/CNF are collected on the MCE filter and transferred to TEM grids following the procedure outlined in NMAM 7402, and then

analyzed using TEM. In addition, NIOSH 5040 method [51] has been used to measure elemental carbon mass concentration for comparison to the REL for CNT and CNF. This method uses a quartz fiber filter with 37 mm sampling cassette for filter based sampling at an air flow of 2 to 4 L/min. The collected sample is analyzed using Evolved Gas Analysis (EGA) by thermal-optical analysis to measure elemental carbon [47,52].

The REL for exposure to ultrafine TiO₂ specifies the use of NIOSH 0600 method to determine mass concentration [48,53]. This method uses a 10-mm nylon cyclone and Polyvinyl Chloride (PVC) filter with 5 µm pore size, operated at 1.7 L/min, to collect particles with a 4 µm median cut diameter for gravimetric analysis. Although filter-based sampling has been in use for more than sixty years, sampling devices using a filter as the substrate for particle collection have been newly designed in the past decade for nanoparticle collection. Other new samplers adopting different substrates including electron microscope grids such as TEM

grid, a combination of grid and filter, or other novel substrates to collect nanoparticles are discussed in this study. Based on the available information to date, personal samplers available for nanoparticles as listed in Table 1 are the personal sampling system PGP, Nanobadge, TEM Partector and Personal Nanoparticle Sampler (PENS), developed in Europe and Asia, and the personal Nanoparticle Respiratory Deposition (NRD), Thermophoretic Nanoparticle Sampler (TPS), Electrostatic Precipitator (ESP) and Tsai Diffusion Sampler (TDS), developed in the US.

The personal sampling system PGP was developed by the Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (IFA) [54]. It is a personal filter holder system for evaluating exposures from hazardous materials. It is used with pre-selector, porous polyurethane foam, for respirable and alveolar size-selective sampling. A gold-coated track-etch membrane filter is used as the substrate to collect particles. A version named PGP-FAP can be used for sampling fibers. It is usually operated at 2 L/min airflow with the filter surface facing downwards, and uses a 30-mm diameter inlet nozzle to keep the face velocity of the filter low [39]. The efficiency varies with the type of filter used in the PGP system.

Nanobadge [55] by Nano Inspect (Alcen Group, Paris, France and French Alternative Energies and Atomic Energy Commission CEA, Grenoble, France) collects particles on a polycarbonate track-etched membrane or quartz filter, which is analyzed offline by X-Ray Fluorescence Spectroscopy (XRF) to provide a mass-based quantification of the chemical elements present on the filters. The filter sample can be further characterized with SEM. The filter unit operates at 0.6 L/min or 1 L/min using a self-contained battery and pump, and is a sealed cassette and equipped with a chip to store data. Nanobadge can be equipped with pre-separators such as impactors to collect size selective particles. Collection efficiency was not reported.

The personal Nanoparticle Respiratory Deposition (NRD) sampler, designed to collect particles smaller than 300 nm, operates at 2.5 L/min and it consists of a 25-mm respirable aluminum cyclone fitted with an impactor (Teflon membrane filter) and a diffusion stage containing mesh screens [56]. The NRD sampler uses an impactor to remove large particles and a diffusion stage where the airborne nanoparticles diffuse to and are collected onto eight hydrophilic nylon mesh screens with 11 μm pore size and 6% porosity. The collection efficiency of the NRD sampler impaction stage ranges from approximately 8% for 100 nm, 26% for 15 nm and approximately

96% for 550 nm particles. The deposition of particles smaller than 100 nm follows the International Commission on Radiological Protection (ICRP) [57] total respiratory deposition. It was designed to be capable for use as a full work-shift personal sampler in a workplace atmosphere. The NRD sampler was limited

in intended use to airborne metal and metal-oxide nanoparticles. The nylon mesh screens can be analyzed with Scanning Electron Microscopes (SEM) for sizing, counting and assessing chemical composition of collected particles on the nylon fibers. The nylon media can be digested for a comprehensive analysis of the particles collected.

Using impaction, a Personal Nanoparticle Sampler (PENS) was designed to collect respirable particulate mass and nanoparticles in workplaces operated at 2 L/min [58]. The PENS use a respirable cyclone and a micro-orifice impactor and collects the nanoparticles on a final filter after the impactor. The final stage is a filter cassette containing a 37 mm Teflon membrane filter. It provides cutoff aerodynamic diameter of 4 μm and 100 nm by cyclone and micro-orifice impactor respectively. Collected particles are measured gravimetrically and can be further analyzed chemically. The collection efficiency of the rotating impaction plate has a maximum value of 94% at 200 nm and declines to 85% at 560 nm. The efficiency is below 50% for particles smaller than 100 nm, and the collection efficiency for particles from 200 nm to 4 μm remains very close to 100% [58]. The PENS is able to sample particles for the 8 h duration of a typical work shift. Operational limitations include a minimal mass concentration of 2 $\mu\text{g}/\text{m}^3$ to ensure the filters can be weighed and the need to prevent the PENS from overloading. PENS were used to sample metalworking operations and measured particle mass concentration (in $\mu\text{g}/\text{m}^3$) for $\text{PM}_{0.1}$, $\text{PM}_{0.1-4}$ and PM_4 for personal exposure and area concentrations [59].

The sampling methods and samplers described above utilize filters as substrates to collect particles and use pre-separators such as a cyclone or impactor to provide size selective sampling. As listed in Table 1, four out of eleven samplers/methods evaluated here use microscope grids to collect nanoparticles. A sampling method using a grid attached polycarbonate membrane filter has been used with a personal sampling cassette to collect nanoparticles and particles in micrometer sizes [36]. Another new sampler named Tsai Diffusion Sampler (TDS) was designed using the geometry, shape of the cassette and operating flow rate to determine cutoff diameter of sampling particles [44]. The TDS is a 25-mm sampling cassette, it uses a filmed copper TEM grid attached to a polycarbonate filter with 0.22 μm pore size, and operates at a low flow rate of 0.3 L/min to collect particles for direct analysis using TEM and SEM. The particles entering the TDS with 50% efficiency has a Mass Median Aerodynamic Diameter (MMAD) of 3.8 μm ; the Count Median Diameter (CMD) of aluminum oxide nanoparticles collected on the filter and on the grid, are 500 nm and 100 nm, respectively. The theoretical combined collection efficiency of the grid and filter is close to 100% for particles entering the TDS [44]. The TDS has been demonstrated for various practical sampling [44]. The filter can also be weighed to obtain mass concentration.

A Thermophoretic Nanoparticle Sampler (TPS) [42] uses thermophoretic force to collect nanoparticles onto a standard TEM grid, and then the characteristics of the sampled nanoparticles can be determined by electron microscope. The TPS is operated at 0.005 L/min air flow (with a programmable set point between 0.001 to 0.01 L/min) and is a stand-alone device containing a micro sample pump and battery that allows up to 8 h of continuous sampling. The sampled grid is analyzed to determine the number and size of the particles collected. A transfer function is also provided that can calculate the number and size distribution of the particles found on the grid. The TPS was tested using sodium chloride (NaCl) particles and the measured CMD was 74 nm. Theoretically, the collection efficiency of TPS decreases with increasing particle diameter [46]. The theoretical collection efficiency ranges from 25% to 20% for particles with diameters from 20 nm to 500 nm [42], but the measured efficiency in this study, described below, is much higher, approximately 60% at 600 nm. The TPS was recently used to monitor particles in the international space station, but experimental results have yet to be published [60]. The TPS has also been used in previous studies [61–63] for nanoparticle sampling, but none has systematically used TEM imaging to characterize the size fractions of particles collected.

The TEM Partector from NANEOS (naneos particle solutions GmbH) in Switzerland, uses Electrostatic Precipitation (ESP) to deposit nanoparticles directly on a TEM grid. It can automatically stop sampling at the optimal grid coverage [41,45]. The sampler is operated by self-contained battery at a flowrate of 0.45 L/min, collects particle sizes from 10 nm to 10 μ m. The sampling efficiency is approximately 3% at 50 nm.

Another sampler designed to collect particles on a TEM grid by ESP was developed by NIOSH and reported by Dixkens in 1999 and later by Miller in 2010 [64,65]. It is meant to collect ultrafine aerosols in workplaces and analyze collected particles using electron microscopy. It is a stand-alone device with a built-in battery and pump, operated at an air flow of 0.055 L/min. It was initially evaluated using sodium chloride (NaCl) aerosols in the 30–400 nm size range. Electron microscopy images of collected particles can be used to estimate particle size distributions. The ESP by Miller [65] has the maximum collection efficiency of NaCl particles approximately 86% with the lowest efficiency in the 100 to 200 nm range. The ESP was designed to collect a representative sample in a timely manner with typical sampling time from few seconds to few minutes. The sampling duration is estimated by calculating the time needed to capture an ideal sample based upon the assumed aerosol concentration and the operating air flow. As an example, sampling for an aerosol of concentration approximately 10^6 particles/cm³ requires only 8s sampling time. It has been used to collect emissions from diesel engines, and Fe-containing aerosols were found through TEM analysis on the collecting grid [66].

Most of the sampling devices and methods described above have been used in various studies to characterize nanoparticles in the field and in the lab [32,34,36,40,42,51,53,61,63,65,67,68]. Based on the functioning mechanisms for nanoparticle collection as interpreted, the practical performance of three devices was studied here.

Experimental Methods

Background

As seen in Table 1, samplers using a TEM grid as a substrate to collect nanoparticles are the latest technique. The samplers available for evaluation at the time of this study were the TPS, TEM Partector, ESP and TDS. These devices rely on three different deposition mechanisms, i.e. thermophoretic force (TPS), electrostatic force (TEM Partector and ESP) and diffusion (TDS) to collect particles. According to the theoretical mechanisms as discussed above, smaller or larger sizes of nanoparticles may be favored differently by one sampling mechanism or sampler than the others for collection. However, practical sampling outcomes evaluating whether a measurable difference can be observed among the different grid samplers have not been published. To address this, experiments have been performed using three different aerosols to study particle collection on grids using available samplers, which include the TPS, ESP and TDS.

Test Aerosols

Three aerosols used were representative of engineered nanomaterials (aluminum oxide), a common aerosol used for laboratory testing (ISO fine test dust), and natural aerosol containing nanometer-sized particles (sodium chloride). Airborne concentrations of each test material were generated using a suitable method. Aluminum oxide (Al₂O₃) powder (Nanophase, Romeoville IL, USA), with a mean primary particle diameter of 40 nm, was dispersed using a compact digital mixer (Cole-Parmer, Vernon Hills IL, USA), which stirred 200 mL of aluminum oxide in a 500 mL Büchner flask and generated airborne Al₂O₃ particles. ISO fine test dust (12103-1 A2), referred to here as road dust, 73% silica with a CMD of 5 to 11 μ m, was dispersed using a Wright Dust Feeder (WDF II, Westwood NJ, USA). Sodium chloride particles were produced using a TSI (Shoreview, MN, USA) particle generator (model 8026) which atomized a saltwater solution and generated airborne particles with a CMD of 40 nm.

Experimental Process and Instruments

Aerosols were generated and released into the center of an ultra-filtered glove box (Terra Universal, Fullerton CA USA, 89 cm x 61 cm x 64 cm) for sampling as shown in Figure 1. Two RTIs and three nanoparticle samplers were positioned equidistant from the aerosol source (Figure 1). Particle concentrations were recorded ten minutes prior to generating aerosols and continued

after test completion. Three repetitions were performed for each aerosol. The filtered glove box air flow was maintained during all tests to provide a constant air exchange. The air velocity at the sampling area ranged from 1 to 7.6 cm/s (2 to 15 ft/min) horizontally and from 0 to 3 cm/s (0 to 6 ft/min) vertically. All tools and devices used inside the glove box were surface cleaned before and after tests.

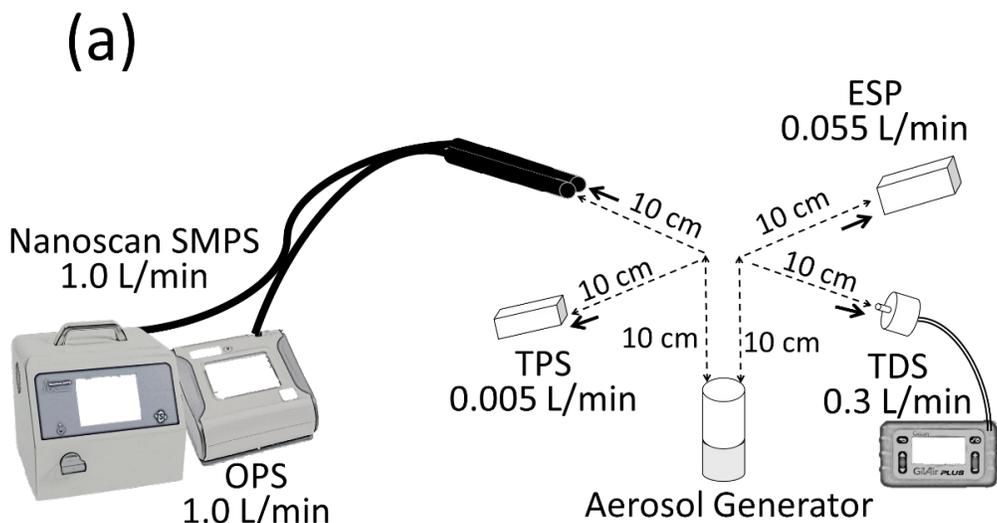


Figure 1a: Experimental setup for sampling aerosols in a filtered glove box. (a) For sodium chloride (NaCl) and road dust,

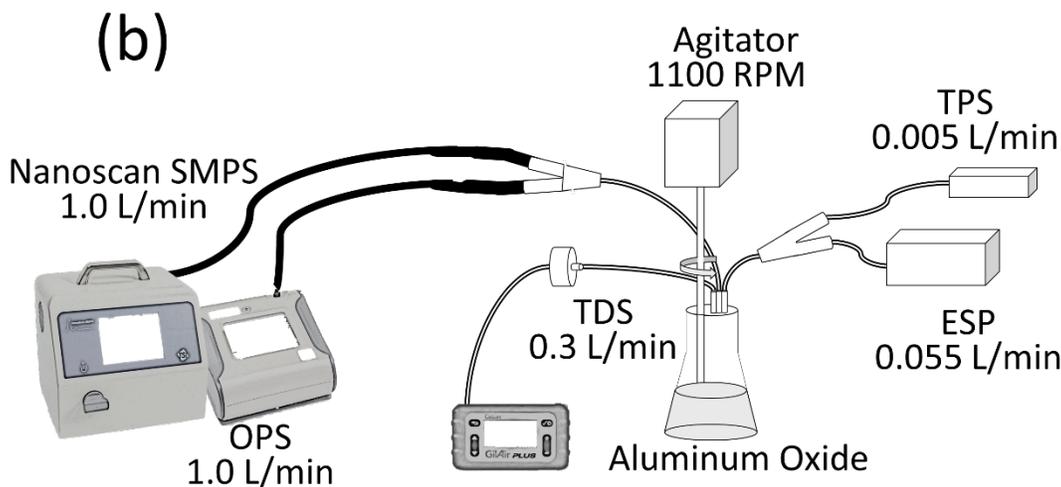


Figure 1b: For aluminum oxide (Al_2O_3) particles. Particle generation and sampling are at the center of the glove box, and Nanoscan SMPS and OPS are placed outside the glove box.

The two RTIs used were a Nano scan Scanning Mobility Particle Sizer (SMPS) (Model 3910, TSI, Shoreview, MN, USA), which measured particle number concentrations with diameters ranging from 10 - 420 nm in 13 size channels, and an Optical Particle Sizer (OPS) (Model 3330, TSI, Shoreview, MN, USA), which measured particle number concentrations with diameters

ranging from 0.3 - 10 μm in 16 size channels. Both instruments had one-minute sampling time for each measurement. Conductive tubing (~90 cm) was connected to each RTI air inlet to reach the aerosol generation source inside the glove box. RTI data were exported and analyzed. For samplers, the TDS and ESP (ESP nano 100, Dash Connector Technologies, Spokane WA, USA) collected

particles on TEM copper grids (400-mesh with carbon coating) and the TPS (TPS 100, RJ Lee, Pittsburg PA, USA) utilized 400-mesh carbon coated nickel TEM grids.

The road dust and sodium chloride aerosols were released into the center of the glovebox 13cm above the work surface. RTIs and personal samplers were positioned 10cm above and 10cm away from the release source as shown in Figure 1. For sampling aluminum oxide, the samplers were attached with short tubing to reach into the opening of a Büchner flask as a stirrer propelled and aerosolized the powder. Previous testing indicated this was an optimal way to get sufficient and stable aluminum oxide concentrations. This set up was at the center area of the glove box and relatively away from the corners and edges. The TPS and TDS collected aerosols for 40 min and the ESP operated for 50 s (recommended time for a 10^5 particles/cm³ maximum concentration).

Electron microscope imaging and data analysis

Particles collected on TEM grids were analyzed using a TEM (Model 2100F, JOEL, Peabody, MA, USA) at 200 kV equipped with a digital Gatan Ultrascan camera. Particle images were taken following a standardized protocol: (1) Low resolution photos (80x) were taken of the center and four corners of the grid to establish if the sample was successful and had enough particles for analysis; (2) Images (500x) of individual grid spaces were taken that represented the range of low to high particle depositions; (3) Grid spaces were taken which showed medium level of particle deposition as representative for detailed imaging; and (4) One or more grid spaces were imaged and methodically traversed (6000-8000x) until 300 particles were analyzed or the grid space was completely imaged.

TEM images were analyzed using FIJI [69] imaging software to count particles and measure diameters. Irregularly-shaped agglomerates are sized by measuring the image area and converting to the equivalent diameter of the equal-area circular particle. Data were transferred to Excel spreadsheets and size fractionated particle counts for each nanoparticle sampler were analyzed. Statistical differences were analyzed using Chi square values calculated using SPSS. P-values (two tailed) were used to validate the 95% confidence level.

Results and Discussion

The total particle number concentrations during the experimental period are shown in Figure 2a and 2b; concentrations

were fairly stable during the sampling period. The average concentration and size distribution for each test aerosol are shown in Figure 2b, which plots particle concentrations from 10 to 420 nm using the concentration scale on the left and particles from 0.3 to 10 μ m on the right y-axis. The test aerosols had different size distributions; road dust contained larger and a wider size range of particles than aluminum oxide and sodium chloride. The generated concentration varied with particle size and the peak concentrations exceeded 10^5 particles/cm³ in 100-200 nm size range. Road dust was found to generate the most particles in micrometer sizes with peak concentration exceeding 5×10^3 particles/cm³.

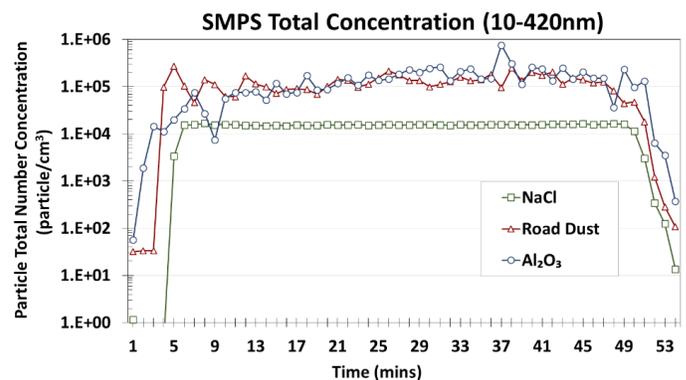


Figure 2a: Particle concentration measurements by real time instrument for three aerosol types with Nanoscan SMPS measures 10 to 420 nm and OPS measures 0.3 to 10 μ m (a) Total particle number concentration of Nano scan SMPS during experiment.

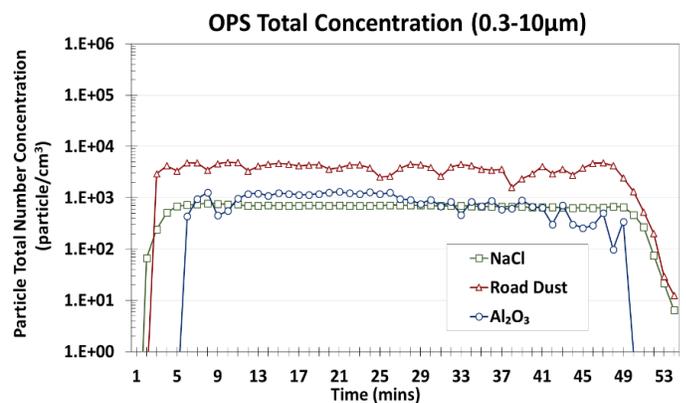


Figure 2b: Total particle number concentration of OPS during experiment.

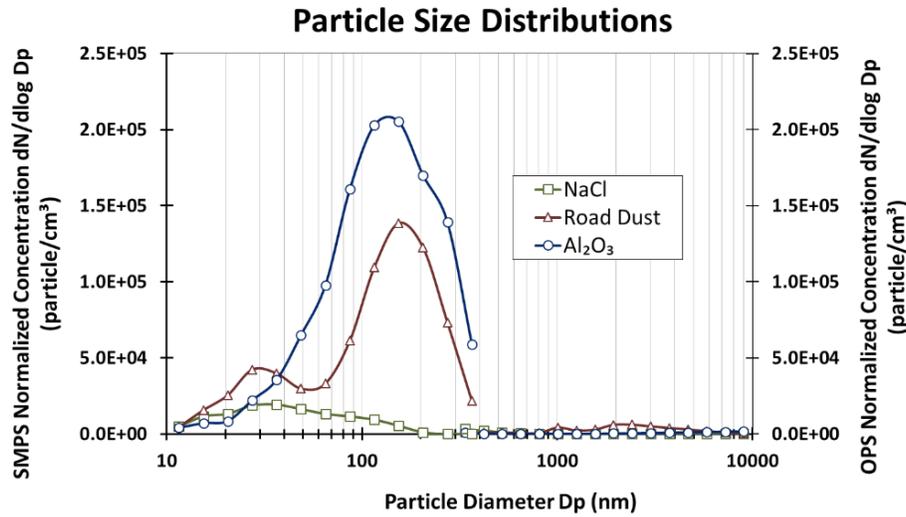


Figure 2c: Average concentrations and size distributions with Nanoscan SMPS data referring to left y-axis and OPS data referring to right y-axis.

The results of the size analysis on particle TEM images for each test aerosol are shown in Figures 3a-3c. Examples of TEM images are shown in Figures 4,5. When sampling the same aerosol, the sizes of particles collected on the grids did not differ from one sampler to another; however, the particle count in some size ranges varied between samplers. Such differences also appeared from test to test with the same sampler, as shown by the standard deviation of each particle count seen in Figures 3a-3c. The aerosol particles counted here include primary particles and small and large agglomerates up to the micrometer size range.

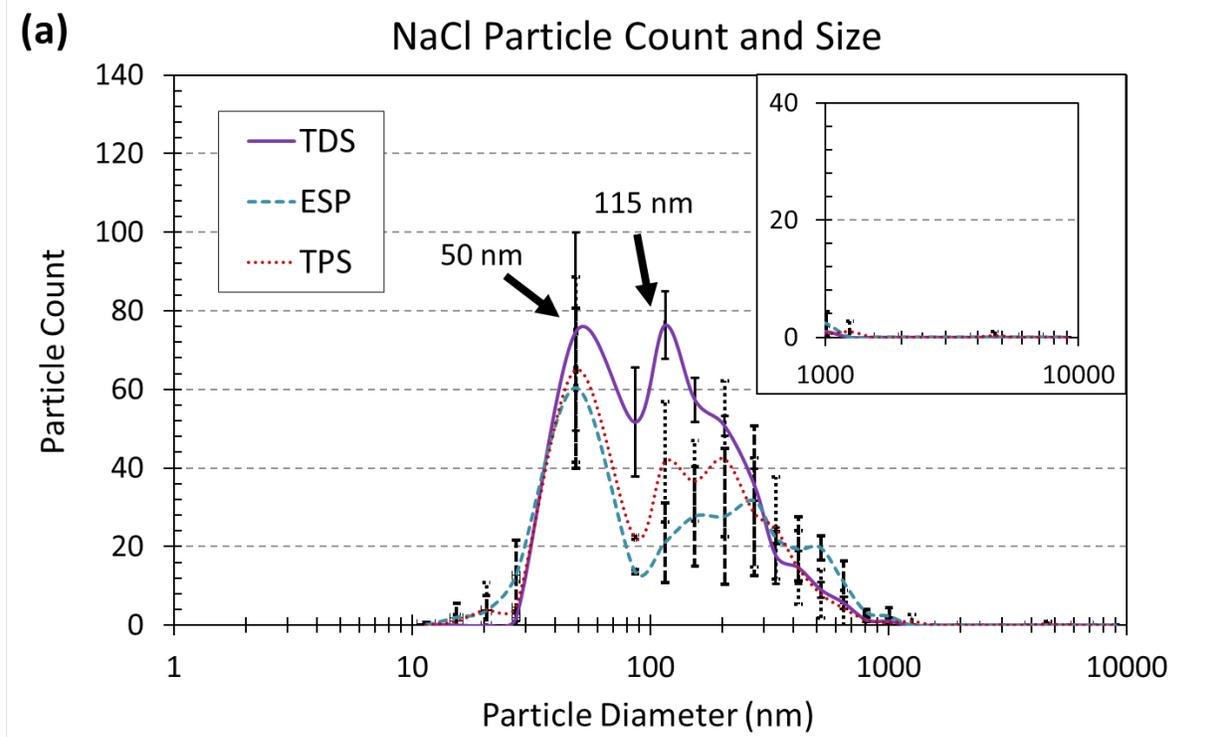


Figure 3a: Particle count and diameter analyzed from TEM images of particles collected by three samplers, TPS, ESP and TDS for (a) NaCl.

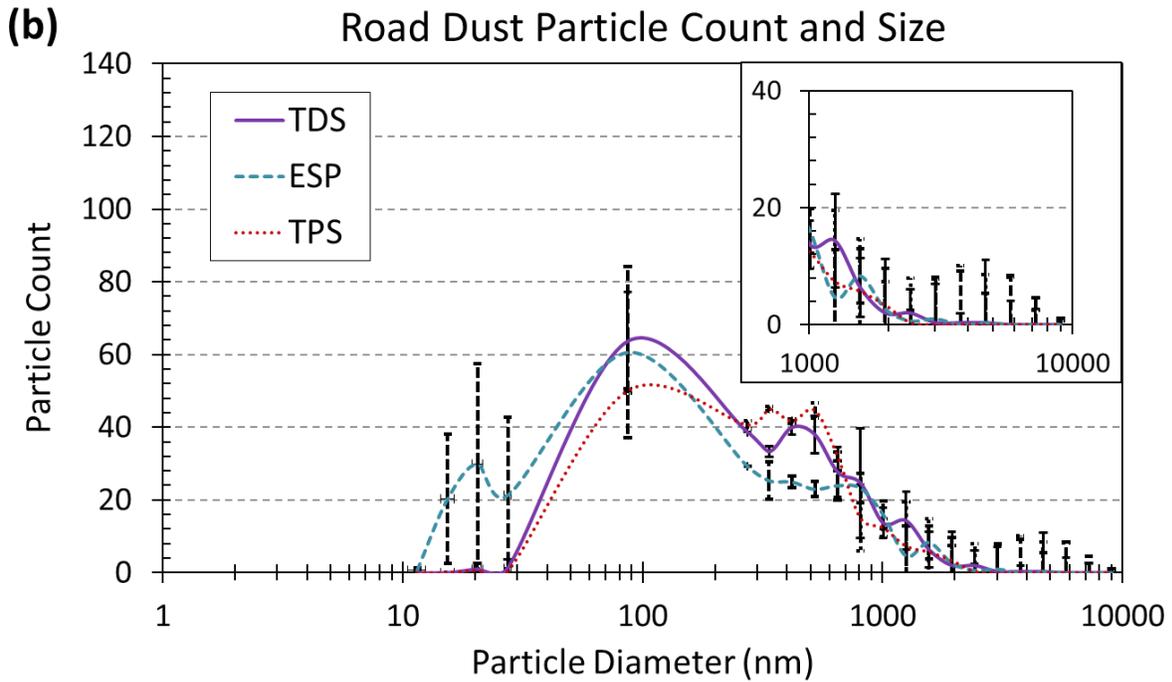


Figure 3b: Road dust.

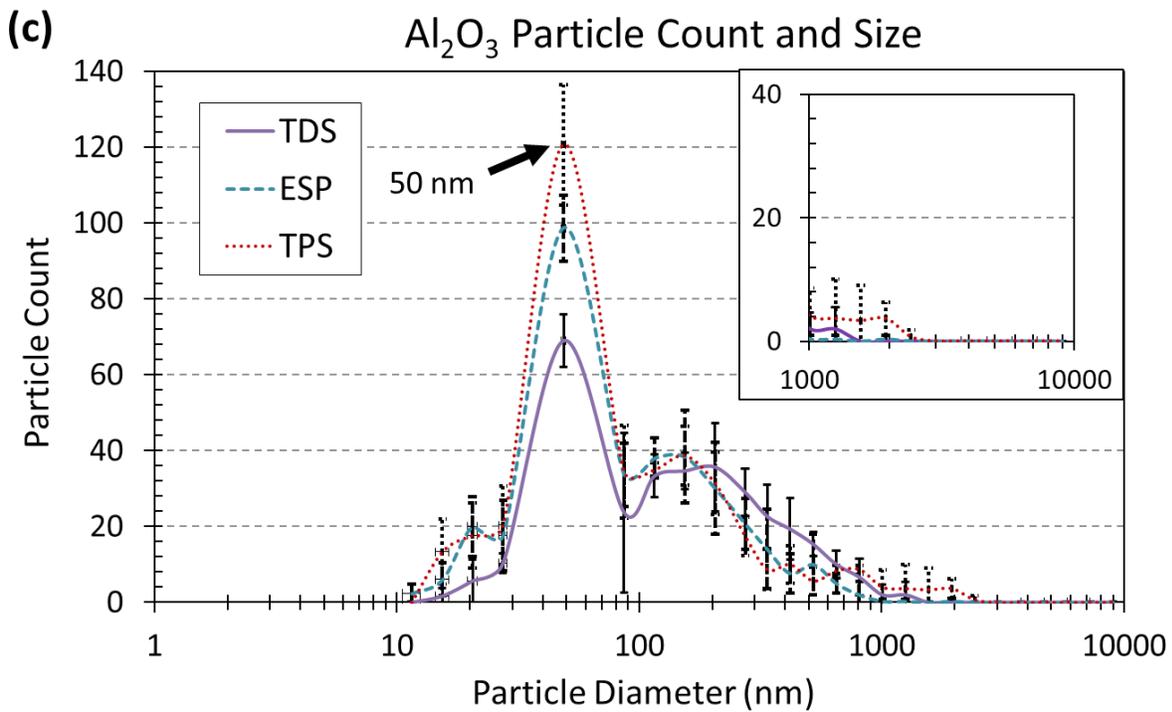


Figure 3c: Al_2O_3 particles.

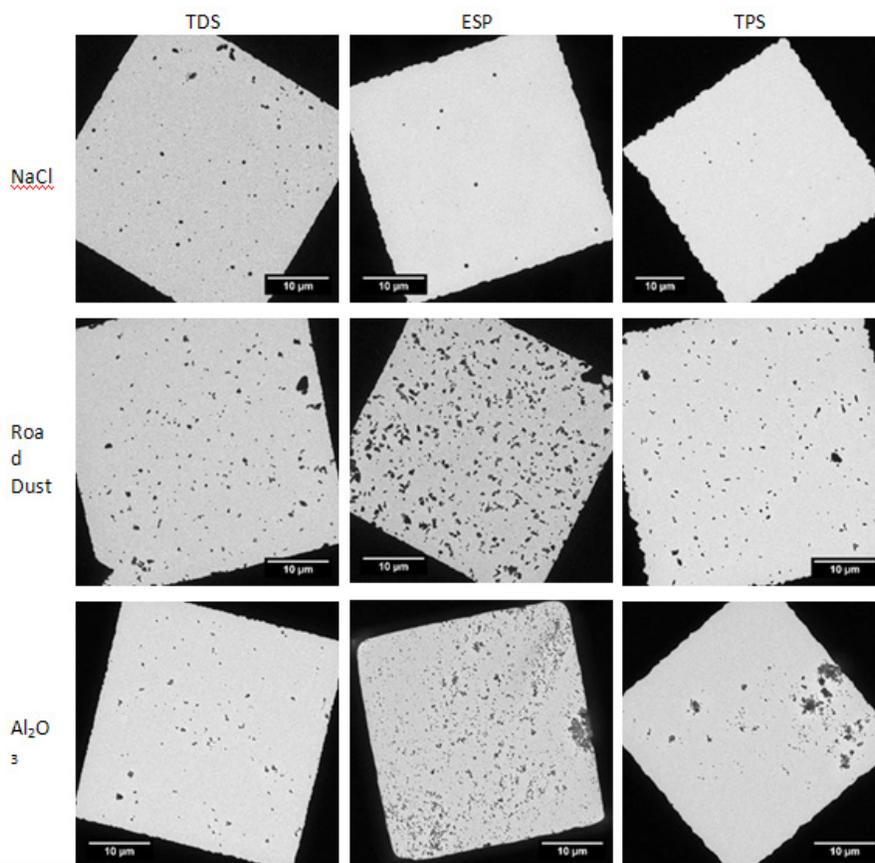


Figure 4: TEM images of collected particles on grid spaces from sodium chloride (NaCl), road dust and aluminum oxide (Al₂O₃) particles using TDS, ESP and TPS. The scale bar of each image is 10 µm.

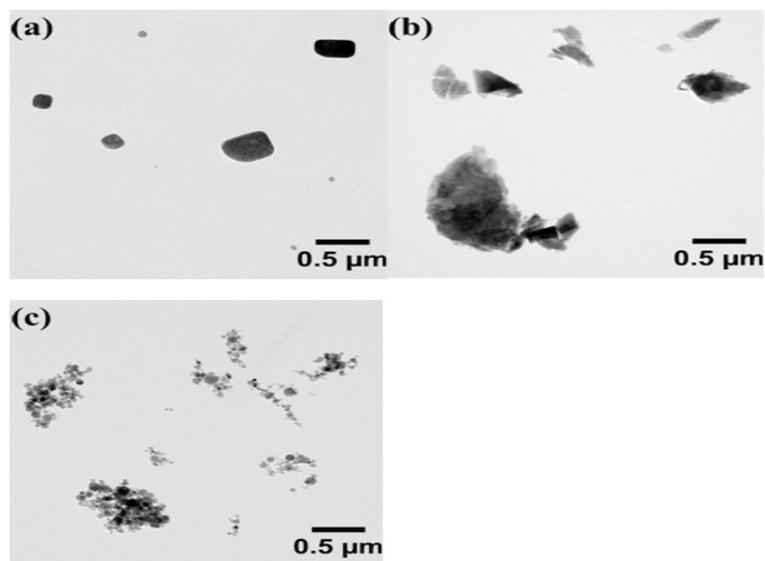


Figure 5: TEM images of different particle types showing morphology and agglomeration: (a) NaCl, (b) road dust, and (c) Al₂O₃

Sampled sodium chloride particles ranged in diameter from 10 nm to about 1 μm (Figure 3a), while the road dust particle diameters ranged from 10 nm to 10 μm (Figure 3b) and aluminum oxide particle diameters ranged from 10 nm to approximately 3 μm (Figure 3c). These size analyses were consistent with the RTI measurement as seen in Figure 2, where road dust particles showed the widest size range, followed by aluminum oxide and then sodium chloride particles which measured as the smallest. Nano particles less than 100 nm were found, and a large portion of particles were counted in this range on all grids regardless of the aerosol or the sampler used. As consistent with an earlier study [70] which utilized sodium chloride particles, the ESP collected sodium chloride particles with highest efficiency in the 30 to 100 nm size range, and above 300 nm; results from this experiment found that the highest particle number count by the ESP was also within the 30 to 100 nm range as seen in Figure 3a. Miller's study reported mean diameters of 40 - 200 nm using a different method of particle generation [65].

The OPS measured a higher concentration of road dust than the Nanoscan SMPS, which is consistent with the particle image count (Figure 3b). However, the mode diameters and particle size distributions of each test aerosol as measured by RTIs were not in good agreement with the size distributions of particles collected by the three samplers. TEM images of particles collected on grid spaces are shown with overviews of single grid spaces in Figure 4, with each image representing a combination of one particle type and one sampler. The primary particle and its morphology of each tested material are shown in Figures 5a-5c. Agglomeration of numerous primary-sized particles is obvious among collected aluminum oxide particles, and road dust, as expected, showed a range of particle sizes. Many of the collected sodium chloride particles were primary particles. All three tested samplers successfully collected a sufficient number of particles on the grid space for a determination of elemental composition using EDX and size analysis utilizing microscope measuring tools and image software.

Since all samplers collected each test aerosol under identical operating conditions, the differences in the size fractions they collected most likely reflect the differences in the mechanisms in which the samplers use to deposit particles onto the TEM grid, as described in the Supplemental Information (SI), the section of Particle Deposition Mechanism. However, the test results for all three test aerosols found that the ESP, TPS and TDS collected samples of similar size ranges. Although the size range was consistent, the size distributions exhibited some differences. For sodium chloride particles (Figure 3a), the three samplers collected a comparable number of particles at the peak size of approximately 50 nm ($p > 0.05$), since the standard deviations mostly overlap each other. The particles with diameters at the other peak size of 115 nm were significantly different between the ESP and TDS ($p < 0.05$)

(Figure 3a). Such differences at 50 nm were not seen on road dust sampling, where larger standard deviations were seen on particles smaller than 30 nm collected by ESP as compared to the other samplers. For aluminum oxide sampling, particles with a peak diameter at 50 nm were measured with significantly different concentrations among ESP, TPS and TDS with 95% confidence level ($p < 0.05$).

According to the samplers' theories of operation (see SI), the diffusion and thermophoretic force should collect smaller particles with the highest efficiency, and the electrostatic force should be most capable of collecting larger particles; however, this difference was not observed here. Particle deposition may be affected by thermal resistance of particles affecting heat transport and electrical resistivity of particles [71-73]. Although the results here demonstrate that all three devices can effectively collect particles in the nanometer size range, additional investigation is needed to more fully understand the effects of various aerosol properties such as electrical or thermal resistance on the different sampler collection mechanisms. This is relevant to exposure assessment as some particle sizes can be under or over represented if a particular sampler favors certain aerosol properties and thus may mischaracterize the magnitude of risk created by certain aerosols.

Since the actual collection efficiency of these devices has not been fully determined experimentally, at this time the results cannot be used to quantify actual particle number concentration as a function of particle diameter. The use of sampling devices to collect particles on grid substrates at this time typically requires the use of RTIs to measure the particle concentration as a reference to determine sampling time to avoid overloading the grid and to have an estimate of the overall particle number or mass concentration. For those devices used for gravimetric sampling, the use of RTIs is not necessary to determine the operation.

In addition, this study did not include the analysis of particles collected on the polycarbonate filter used in the TDS, which could be analyzed with a SEM to measure the diameter, shape, and elemental composition of larger micrometer-scale particles. One advantage of the TDS compared to the ESP and TPS is that it theoretically collects close to 100% of the particles on the grid and filter, so that the proper combination of particle counting on the grids via TEM and the filter via SEM should give a complete size distribution.

The finding that the Nanoscan SMPS peak concentration did not always match particle mode sizes collected by the samplers may have been due to differences in how the particles were measured. The Nanoscan SMPS measured electrical mobility particle size which is a function of the surface area of irregularly-shaped particles and agglomerates while the samplers collected particles by diffusion, thermophoresis and electrical mobility and

utilized TEM imaging which measured particle two-dimensional projected area diameter. Given these differences, such variability is not surprising.

Conclusions

This study reveals that all of the methods evaluated have advantages and disadvantages; at this time, available data can point to no single method that can be considered “ideal”. The data presented here indicate that collecting nanoparticles directly onto grids for TEM analysis can be an effective technique for performing qualitative analysis regarding particle identification and elemental analysis. This study demonstrates for the first time that quantitative analysis of sampled aerosols on grids can be performed to obtain particle size distributions in the nanometer size range. Results from this study present that the studied sampling and analytical techniques can provide reliable information to interpret the exposure level and associated health effects.

Further research is needed in at least two areas. First, since most real-world nanoparticle aerosols are present in size distributions that include larger particles, research is needed to extend the particle range measured for microscopic analysis. Second, even when a particle size distribution can be determined with some accuracy, the actual number concentration of the aerosol must be determined. This depends on knowing the actual particle collection efficiency of each device; determining this will require further experimentation. The work reported here indicates that nanoparticle sampling methods that deposit particles directly onto TEM grids show great promise, and deserve further research toward the goal of developing effective, quantitative airborne nanoparticle sampling methods.

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