In vitro toxicity evaluation in A549 cells of diesel particulate matter from two different particle sampling systems and several resuspension media

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Abstract
In urban areas, inhalation of fine particles from combustion sources such as diesel engines causes adverse health effects. For toxicity testing, a substantial amount of particulate matter (PM) is needed. Conventional sampling involves collection of PM onto substrates by filtration or inertial impaction. A major drawback to those methodologies is that the extraction process can modify the collected particles and alter their chemical composition. Moreover, prior to toxicity testing, PM samples need to be resuspended, which can alter the PM sample even further. Lastly, the choice of the resuspension medium may also impact the detected toxicological responses. In this study, we compared the toxicity profile of PM obtained from two alternative sampling systems, using in vitro toxicity assays. One system makes use of condensational growth before collection in water in an impinger – BioSampler (CG-BioSampler), and the other, a Dekati® Gravimetric Impactor (DGI), is based on inertial impaction. In addition, various methods for resuspension of DGI collected PM were compared. Tested endpoints included cytotoxicity, formation of cellular reactive oxygen species, and genotoxicity. The alternative collection and suspension methods affected different toxicological endpoints. The water/dimethyl sulfoxide mixture and cell culture medium resuspended particles, along with the CG-BioSampler sample, produced the strongest responses. The water resuspended sample from the DGI appeared least toxic. CG-BioSampler collected PM caused a clear increased response in apoptotic cell death. We conclude that the CG-BioSampler PM sampler is a promising alternative to inertial impaction sampling.

KEYWORDS
apoptosis, diesel exhaust, extraction, impinger, particulate matter, reactive oxygen species, sampling, soot, toxicity
**1 | INTRODUCTION**

Combustion produces large quantities of fine particles in both indoor and ambient air pollution, which are thought to cause significant adverse health effects worldwide (Thangavel et al., 2022; WHO, 2003). Diesel engines in particular have been identified as major sources of harmful particulate matter (PM) in urban areas (Karagulian et al., 2015). Exposure to diesel exhaust has been linked to a wide spectrum of adverse health effects, including morbidity and mortality (U.S. EPA, 2002).

Diesel combustion derived particle exposure studies using different in vitro cell types have revealed cytotoxic, genotoxic, inflammatory, and oxidative stress effects (Bayram et al., 2006; Dybdahl et al., 2004; Jalava et al., 2010; Rao et al., 2005; Zerboni et al., 2022). In vitro cell systems utilizing toxicological tests on diesel engine particles (DEP) rely on the availability of relatively large quantities of PM mass, usually tens of milligrams (Kumar et al., 2021).

PM sampling is typically conducted through air filtration and/or inertial impaction methods, which capture PM particles on solid substrates. However, for in vitro toxicological studies, the PM needs to be separated from these substrates using various extraction techniques with different solvents. This process can sometimes result in incomplete extraction of PM or insoluble components (Dahe et al., 2011; Willeke et al., 1998).

For instance, it can be problematic to coextract fat- and watersoluble species such as semivolatile organic compounds (SVOC) and inorganic ions such as ammonium nitrate with a single choice of solvent (Bein & Wexler, 2015; Eaton et al., 2003). In addition, extraction of soot from substrates is usually not complete. Another drawback that has been observed is the changes in chemical speciation (e.g., shifts in oxidation state of redox active metals) during extraction (Eaton et al., 2003; Turpin et al., 1994). Many of the known uptake mechanisms, along with physical and chemical clearance processes that take place in the epithelium of the respiratory tract are dependent on size, composition and surface chemistry of the PM (Nel et al., 2006; Oberdörster et al., 2005). Ideally, to preserve the original composition, the choice of collection and sample preparation method should have as little impact on these properties as possible.

One alternative to traditional PM collection methods is direct collection into liquid media using bioaerosol impingers (Kim et al., 2001; Wang et al., 2013). In this technique, aerosolized particles are led into a swirling liquid and then collected by a combination of impaction and centrifugal forces. Efficient collection of the smallest particle fraction in a BioSampler relies on particle growth, which can be achieved using systems for condensational growth (CG) in a saturated humid atmosphere (Geller et al., 2005; Kim et al., 2001; Sioutas et al., 1999; Wang et al., 2013). Benefits of direct PM collection methods are, for example, minimized uncertainties related to PM extraction. Indeed, PM is already in a biocompatible suspension, and therefore, no separate resuspension methods are needed. In addition, PM growth by water vapor along with the inertia-based collection in a BioSampler resemble potential particle growth and deposition in the human lung better than filtration or inert impaction collection. While the collection system based on condensational growth and liquid impingers have shown comparable results with PM collection onto filter substrates regarding PM chemistry (Dahe et al., 2011; Wang et al., 2013), an extensive comparison against a broad spectrum of toxicological endpoints has not previously been reported.

The aim of the present study was to compare toxicological endpoints from PM-samples collected by impinger and filtration making use of a CG-BioSampler system and Dekati® Gravimetric Impactor (DGI), respectively. Tested endpoints included cytotoxicity, formation of cellular reactive oxygen species (ROS), production of inflammatory mediators, and genotoxicity. In addition, for the DGI collected diesel exhaust particles (DEP), different methods for resuspension were also evaluated.

**2 | MATERIAL AND METHODS**

**2.1 | Engine and used fuel**

The diesel exhaust PM was produced using a heavy-duty direct injection diesel engine (Volvo TD45, 4.5 L, 4 cylinders, manufactured in year 1991) meeting the particle emission class EU Stage I and Tier 1 (0.54 g/kWh for PM). The engine was attached to an engine dynamometer and was operated repeatedly using the European Transient Cycle (ETC) with minor modifications. In this study, only the urban part of the ETC cycle was used and is therefore referred to as modified ETC cycle. Low sulfur SD10 (RF-06-03) fuel was purchased from Preem (Preem AB, Stockholm, Sweden). Prior to collection of DEP using both methods, the exhaust was diluted with air (~1:6) (see Figure 1).

**2.2 | CG-BioSampler collection of diesel exhaust particle (DEP) subject to toxicological studies**

DEP subject for in vitro testing were sampled using a custom-designed collection system based on saturation-condensational growth together with Bioaerosol impingers (BioSampler®, SKC inc, Eighty-Four, PA, USA). Figure 1 gives a schematic overview of the collection system. Sampling systems of this type have been shown to allow efficient collection of both ultrafine (<0.1 μm) and fine (0.1–1.0 μm) particles and preserve the physical and chemical characteristics of the sampled aerosols when concentrated into a biocompatible liquid medium (Kim et al., 2001; Wang et al., 2013). Diluted exhaust gas with a mean temperature of 40°C was drawn through the humid headspace above the surface of ultrapure water (Milli-Q® Advantage A10, Merck Millipore, Germany) maintained at 55 ± 2°C, to saturate the gas stream. The water level was controlled regularly by inspecting an auxiliary column and typically refilled two times per sampling period. The particle-vapor mixture then entered a condensation tube connected to a circulating chiller (50:50 ethylene glycol: H2O, LTC 20–40, Grant Instruments Ltd., UK) maintained at −2°C to promote condensational particle
growth. The dimensions of the saturator chamber and the condensation tube resulted in residence times of 3.4 and 0.34 s, respectively, which are close to residence times obtained in similar constructions (Geller et al., 2005; Kim et al., 2001; Sioutas et al., 1999). Furthermore, a good compliance was also obtained for the gas temperature decrease (10 ± 1°C) through the condensation tube. From the top of the condensation tube the exhaust stream was connected to a six-port manifold leading to six Biosamplers®, each filled with 20 mL water. Downstream of each Biosampler® a back-up Polytetrafluoroethylene (PTFE) filter was mounted in an in-line metal holder. The individual Biosampler® flows (12.5 L/min/sampler) provided by pumps (Model DOA-P109-FD, Gast Manufacturing, Inc., MI, USA) were regularly checked with a primary flow meter (Defender 520, Mesa Labs, LA, USA) and back-up filters were replaced when a 10% decrease in the flow was observed. The total air volume sampled through each Biosampler® was measured by gas meters placed downstream of the pumps. Temperatures were monitored in the incoming diluted exhaust and upstream and downstream of the condensation tube (see Figure 1).

Upstream of the water bath, DEP were collected at a flow of 7.0 L/min on filter substrates (Zeflour™, 47 mm, 2.0 μm, Pall Corp., NY, USA) for determination of particle mass concentration. In a subsequent exhaust dilution step, particle number size distributions were measured using a differential mobility particle sizer (DMPS).

2.3 | Impactor sampling of DEP subject to toxicological studies

Impactor collections were performed using a DGI at a flow of 70 L/min. The DGI includes four impaction stages and a backup filter, which classify particles into size ranges: coarse (PM>2.5, aerodynamic diameter Da > 2.5 μm, with cyclone PM10–2.5, Da between 10 and 2.5 μm), inter-modal (PM2.5–1, Da between 2.5 and 1 μm), accumulation (PM1–0.5, Da between 1 and 0.5 μm and PM0.5–0.2, Da between 0.5 and 0.2 μm), and ultrafine (PM0.2, Da < 0.2 μm) particles. Hydrophobic 47 mm PTFE membrane filters (FSLW04700, Millipore) with pore size of 3 μm were used as impaction substrates and 70 mm filters with a similar pore size (FSLW09025, Millipore) as backup filters.

2.4 | CG-BioSampler system collected PM sample handling

PM sampling was performed daily for three consecutive days using the CG-BioSampler system. The six Biosampler® particle suspensions from each sampling day were combined (~120 mL) and stored at 4°C until further processing. The corresponding back-up filters were individually probe sonicated (Vibracell™, Sonics & materials inc., CT, USA)
until the filter was thoroughly stripped from particles, typically three times for 1 min in a beaker using 3 × 5 mL MeOH. The extraction solvents were combined and concentrated to ~1 mL in a rotary evaporator (Rotavapor R-114, BÜCHI Labortechnik AG, Switzerland), before addition of 2 mL MilliQ-water and subsequent concentration to ~2 mL. This concentrated back-up filter portion was then combined with the main Biosampler® suspension, which formed stock sample. The mean DEP concentration in final suspensions from the three sampling periods was 2.1 ± 0.8 mg/mL.

CG-BioSampler system collected PM stock samples were prepared for the cell experiments by first sonicating the stock for 30 min in an ultrasonic water bath before dividing the sample into individual glass tubes. Aliquoted samples were stored at −20°C. When CG-BioSampler samples (Diesel₀) were used in in vitro experiments, the aliquoted samples were thawed for 30 min at room temperature and then sonicated for 30 min in an ultrasonic water bath prior to cell exposure. At this stage, the PM concentration of the CG-BioSampler collected sample was 2.1 mg/mL. A flow chart for sample handling is given in Data S1.

### 2.5 DGI collected PM sample handling

DGI collected filter samples were stored at −20°C immediately after the sampling. All the DGI collected sample substrates were weighed before and after the sampling using an analytical balance to determine PM mass in the filters. The DGI collected particulate material of PM₁ size range was extracted twice with 50 mL of methanol in an ultrasonic water bath for 30 min below 35°C to remove particles from the substrate. The extracts were pooled together and the excess methanol was evaporated using a rotary evaporator at 35°C under pressure. Finally, the particle suspension was dispensed into glass tubes on a mass basis and the samples were dried under a flow of nitrogen (99.5%). The dried particulate samples were stored at −20°C.

Prior to cell exposures, aliquoted samples were thawed for 30 min at room temperature. DGI particulate samples were either suspended into a small amount of dimethyl sulfoxide (DMSO) (20 μL/1 mg PM) and sterile water (W1503, Sigma-Aldrich Corp., St. Louis, MO, USA) (Diesel₀ +w sample), sterile water only (Diesel₀ sample), or in cell culture medium Dulbecco’s Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 100 U/mL penicillin/streptomycin (all Sigma Aldrich, USA) (Diesel₀ sample), all at a concentration of 5 mg/mL. Thereafter, samples were stirred with a glass rod and sonicated for 30 min in an ultrasonic water bath before cell exposure. At this stage, PM concentration of the DGI collected sample was 5 mg/mL and DMSO concentration was 10%. Flow chart for sample handling is described in Data S1.

### 2.6 Cell culture and experimental setup for toxicological studies

A549 (American Type Culture Collection) cells were routinely maintained in DMEM with 10% FBS, 2 mM L-glutamine and 100 U/mL penicillin/streptomycin (all Sigma Aldrich, USA). When cells were sub-cultivated or divided in experiment plates, the cell layer was rinsed with Dulbecco’s phosphate buffered saline (D-PBS) (Sigma Aldrich, USA) before the addition of 2 mL of Trypsin–EDTA solution (Sigma Aldrich, USA). Cells were counted and seeded at a concentration of 150,000 cells per well in 12-well plates for the exposure experiments. After an attachment phase of 24 h, the culture medium was replaced (1 mL) and the cells were left to acclimatize for 1 h. During this acclimatization period, the PM samples were prepared.

The cells were then exposed to four increasing particulate concentrations, that is, 30, 75, 150, and 220 μg/mL (corresponding doses as per surface area are 8.6, 21.4, 42.9, and 62.9 μg/cm²), for 24 h at 37°C and 5% CO₂ in a humidified incubator. The pipetted volumes of the prepared DGI collected PM samples were as follows 6, 15, 30, and 45 μL and for the CG-BioSampler collected sample 11, 28, 56, and 83 μL to achieve the desired concentrations in the cell culture dish. All experiments contained untreated (UN, cell culture medium), water (W, adding 83 μL of W1503 water into cell culture medium), DMSO (Adding 45 μL of 10% DMSO in W1503 water to cell culture medium) and appropriate toxicity assay specific positive control (see Section 2.7). All controls are presented in Data S2. PM doses were selected according to previous experiments (Jalava et al., 2010, 2012).

After the 24 h exposure period, the culture medium was collected and frozen at −80°C for later analysis of the cytokines IL-6 and IL-8. The cells were then washed using D-PBS and detached from the bottom of the wells using trypsin–EDTA. Trypsin activity was inhibited upon the addition of FBS at final concentration of 10%. Aliquots of the cell suspension were taken for analysis of (3-[4,5-dimethylthiazolyl-2]-2,5-diphenylnitrozolium bromide)-test (MTT). The remaining cells were used in cellular ROS assay, comet assay, cell cycle analysis, and annexin V assay.

### 2.7 Analysis of toxicological endpoints

#### 2.7.1 Analyses of inflammation

The pro-inflammatory cytokine IL-6 and the chemokine IL-8 were immunochemically analyzed in the cell culture medium, using commercial enzyme linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA). TNF-α (5 ng/mL) was used as a positive control.

#### 2.7.2 Analyses of cell viability

Cell viability was measured using the MTT assay. The absorbances were measured at a wavelength of 570 nm in a multi-label plate reader and the results were normalized so that exposed cells were compared with control cells (Mosmann, 1983). Any potential interference of the particulate samples with the method was also tested and ruled out. Hydrogen peroxide (13 mM) was used as a positive control.
2.7.3 | Analysis of apoptotic and necrotic cell death using Annexin V and 7-ADD

Analysis of apoptotic and necrotic cell death was carried out using APC Annexin V (Thermo Fisher Scientific) and 7-ADD (Sigma Aldrich). After harvesting, cells were centrifuged (3,200 rpm, +4 °C) and the supernatant was removed. Cells were resuspended in 1× annexin binding buffer (Thermo Fisher Scientific) at a concentration of 1 × 10^6 cells/mL. The cell suspension (100 μL) was incubated with 2 μL of APC Annexin V at room temperature for 15 min in the dark. After that 300 μL of annexin binding buffer (1×) was added to the cell suspension. Next, 1 μL of 7-ADD was added at a final concentration of 2.5 μg/mL. Thereafter, samples were analyzed without delay by flow cytometry (BD Accuri) monitoring FL-3 and FL-4 channels. Data for 20,000 events per sample were analyzed. Staurosporine (0.05 μM) was used as a positive control for Annexin V assay.

Three different cell populations were discriminated within the cell gate: early apoptotic cells that expressed red fluorescence (Annexin+/7-ADD−), late apoptotic/necrotic cells that were positive for both Annexin V-APC and 7-ADD (Annexin+/7-ADD+), and necrotic cells that expressed orange fluorescence (Annexin−/7-ADD+).

2.7.4 | ROS analysis

Cellular ROS formation was assessed from cell suspension aliquots. Cells were centrifuged at 3200 rpm, +4 °C, and the supernatant was then discarded and the cell pellet was resuspended in 220 μL of D-PBS (Sigma). The cells were then plated as 2 × 100 μL aliquots in 96-well plates and 8 μL of 2′,7′-dichlorodihydrofluorescein diacetate solution (H2DCFDA) (0.5 μM in DMSO) was added. The cells were incubated for 30 min at 37 °C before assessing the dichlorofluorescein fluorescence at 485 nm excitation and 530 nm emission. H2O2 (13 mM) was used as positive control for H2DCFDA assay.

2.7.5 | SubG1 and cell cycle

The cell cycle phases and subG1 phase were measured using a flow cytometric DNA content analysis after staining with propidium iodide (PI). The analysis detects the number of cells in the different phases of the cell cycle. The cells were fixed with ethanol (70% v/v) and stored at +4 °C for at least 24 h before analysis. The ethanol fixed cells were separated by centrifugation (10 min, 400 × g) and resuspended into D-PBS. After treatment for 1 h with ribonuclease A (0.15 mg/mL), PI (8 μg/mL) was added to the mixture (all Sigma). The incubation was continued in the dark at +37 °C for 2 h. A total of 12,000 cells were analyzed per sample at an emission wavelength of 613 ± 20 nm by flow cytometer (BD Accuri). Etoposide (1.25 μM) was used as a positive control.

2.7.6 | Analysis of genotoxicity

DNA damage was assessed in the alkaline version of the comet assay as described earlier by Tice et al., 2000. The nuclei were analyzed in ethidium bromide-stained cells (100 nuclei per dose; using an image analysis system [CASPLab v. 1.2.3b2]). The Olive tail moment ([tail mean – head mean] × tail% DNA/100) was the parameter used in the statistical analysis. Benzo(a)pyrene (0.1 μM) was used as a positive control.

2.8 | Statistical analysis

All cell experiments were done using at least four independent replicates. The measured responses were compared with the control and between particle doses. UN control was used in statistical analysis for all samples because it did not differ statistically from other solvent controls except in IL-8 measurement. The data were analyzed in IBM SPSS Statistics 21 (SPSS Inc., Chicago, IL, USA). The results from the toxicological endpoints were evaluated by the non-parametric Mann–Whitney U test. Differences were considered to be statistically significant at p < 0.05. In figures, an asterisk indicates statistical significance between treatment and untreated control value. The lowercase letters in figures indicate a statistically significant response between samples. Letters are given as follows: Dieselp is a, Dieselw is b, Dieselm is c, and diesel_d-w is d. In figures, dashed line represent UN control.

3 | RESULTS

3.1 | Cellular metabolic activity and necrotic cell death

All four samples showed a concentration-dependent decrease in the cellular metabolic activity detected with MTT test (Figure 2A), but at varying degrees. Only Diesel_d-w, however, reached a statistical significance at the highest dose. Similarly, when cell membrane integrity was evaluated using 7-ADD (Figure 2B), only the Diesel_d-w sample had a statistically significant response when the result was compared with untraded cells. Moreover, highest dose of Dieselm differed statistically significantly from the Dieselp sample. Interestingly, the highest concentrations of Dieselm also caused a significantly greater response than that of both Dieselp and Dieselw in regard to cell membrane integrity.

3.2 | Apoptotic cell death

When responses were compared with untreated cells, a statistically significant and concentration-dependent increase in the number of apoptotic cells was detected in cell cycle analysis (sub-G1 phase) after exposure to the diesel particles from both Dieselp and Dieselw.
samples (Figure 3A). Lowest and non-statistically significant responses were seen from Dieselw and Dieselm at least when responses were compared with untraded cells. However, Dieselp and Dieselm differed statistically significantly from Dieselp and Dieselw +w samples. Similar responses were observed when apoptosis was assessed using annexin V expression (Figure 3B), with the exception that there was no statistically significant difference between Dieseld +w and untreated control. Nevertheless, there was a statistically significant difference between the Dieselp and the Dieselm and Dieseld +w samples.

### 3.3 Genotoxicity

All the studied particulate samples induced a concentration dependent DNA damage in the comet assay when compared with control cells, apart from the Dieselw sample (Figure 4). Dieselp, Dieselm, and Dieselw +d samples did not statistically significantly differ from each other. However, the highest PM concentration in all the samples differed statistically significantly from the Dieselw sample.

FIGURE 2 Cellular metabolic activity assessed with the MTT test (A) and cell membrane integrity (B) after 24 h exposure to PM samples from diesel. Bars represent four concentrations (30, 75, 150, and 220 mg/mL); whiskers are the standard error of mean (SEM). Dashed line represents untreated control. The asterisks indicate statistical significance compared with the control ($p < 0.05$) analyzed by the nonparametric Mann–Whitney U test. The lowercase letters indicate a statistically significant response between samples ($p < 0.05$) based on a Mann–Whitney U test.

FIGURE 3 Cells in subG1 phase (A) and Anexin V positive cells (B) after 24 h exposure to diesel particles. Bars represent four concentrations (30, 75, 150, and 220 mg/mL); whiskers are the standard error of mean (SEM). Dashed line represents untreated control. The asterisks indicate statistical significance compared with the control ($p < 0.05$) analyzed by the nonparametric Mann–Whitney U test. The lowercase letters indicate a statistically significant response between samples ($p < 0.05$) based on a Mann–Whitney U test.

FIGURE 4 Detected genotoxic response in A549 cells measured by comet assay after 24 h exposure to diesel particles. Bars represent four concentrations (30, 75, 150, and 220 mg/mL); whiskers are the standard error of mean (SEM). Dashed line represents untreated control. The asterisks indicate statistical significance compared with the control ($p < 0.05$) analyzed by the nonparametric Mann–Whitney U test. The lowercase letters indicate a statistically significant response between samples ($p < 0.05$) based on a Mann–Whitney U test.
3.4 | ROS production

The intracellular formation of ROS by the diesel particulate samples is shown in Figure 5. All of the samples produced statistically significant increases in ROS production in A549 cells when the responses were compared with UN control. Diesel$_m$ and Diesel$_{d+w}$ generated the greatest response. Moreover, the highest dose from those samples differed significantly from the corresponding Diesel$_p$ and Diesel$_w$ dose.

3.5 | Production of inflammatory mediators

Diesel$_p$ and Diesel$_m$ particulate samples triggered a concentration-dependent and statistically significant production of the pro-inflammatory mediator IL-8 in A549 cells (Figure 6A). The highest concentration (220 mg mL$^{-1}$) of Diesel$_w$, and second highest (150 mg mL$^{-1}$) from Diesel$_p$ evoked responses, which were statistically significantly different from the other samples. IL-6 responses followed the same trend as IL-8, although a significant production of IL-6 was also seen from the highest dose of the Diesel$_w$ sample (Figure 6B).

4 | DISCUSSION

The purpose of this study was to compare toxicological responses of PM samples, generated from a heavy-duty diesel engine and collected using two different methods. In addition, three different methods for resuspension of inertial impaction collected DEP were explored. The human alveolar cell line (A549) was used as model system. Extracted DGI samples were resuspended in different media, either pure water, a cell culture medium or a water/DMSO mixture. The CG-BioSampler samples were collected in ultra-pure water. After a 24-h exposure, several toxicological endpoints were evaluated. The data indicated that particles resuspended in the water/DMSO mixture and cell culture medium produced the most pronounced responses, followed by CG-BioSampler sample. Interestingly, the CG-BioSampler collected PM was the only sample that caused a clear response in both apoptotic cell death measuring assays. It is also interesting to notice that the CG-BioSampler PM sample, which was also collected in water, was significantly more toxic than the water-suspended sample (Diesel$_w$, Diesel$_p$) regarding apoptotic cell death, genotoxicity and inflammatory endpoints. This suggests that the resuspension of the PM affects toxicity. The CG-BioSampler collection method might catch certain compounds that the DGI collection method misses. This could make the PM collected using CG-BioSampler more toxic.
Moreover, when comparing our ROS responses to the literature, partially similar results were reported by Jalava et al. (2010), who found that pure petro-diesel particles induced a concentration dependent generation of ROS in a mouse macrophage cell line. Finally, DEP’s ability to induce inflammation seems to be in good agreement with other studies, where low to moderate inflammation was reported (Hemmingsen et al., 2011; Jalava et al., 2012; Wong et al., 2022).

The results regarding resuspension methods can probably be attributed to differences in medium suspension efficiency of PM and its chemical constituents. When soot particles are mixed with water, they tend to agglomerate, ending up with a reduced overall surface area. Indeed, surface area of the DEP seems to play major role in induced toxicity, as seen in correlation studies done after mice were exposed to a single intratracheal instillation of diesel particles from various sources (Bendtsen et al., 2020). In that study, diminished surface area of particles led to reduced toxicity. This may relate to the Dieselw sample in the present study. To avoid agglomeration, DMSO is usually added during resuspension of the sample. This treatment appeared to increase the toxicity of the particles when comparing a water suspended sample, as shown clearly by increased genotoxicity. However, DMSO may potentially react chemically with the studied material or affect cell functions (Rammler & Zaffaroni, 1967; Santos et al., 2003), which could also contribute to the observed increase in toxicity. In addition, DMSO has been shown to induce anti-inflammatory responses (Santos et al., 2003) that may have contributed to the detected differences in the release of inflammatory mediators between the cell culture medium and water/DMSO mixture of resuspended particles. There is also a potential risk that DMSO would remove soluble components from the DEPs, resulting in less harmful particles entering the cell membrane (Kim et al., 2018).

When we resuspended PM using cell culture medium, similar results were detected as with the water/DMSO mixture with some exceptions in detected cell death and inflammation responses. Cell culture medium containing serum can probably form a protein corona surrounding diesel particles (Shaw et al., 2016) and, thus, prevent aggregation, explaining similarity in responses compared with Dieselw sample. Protein corona formation and beneficial nanosize particle suspension have been studied extensively with engineered nanoparticles (Capjak et al., 2017; Neagu et al., 2017). Moreover, this suspension method more closely mimics the real situation in the human lung, where proteins in the respiratory tract lining fluid are present and can bind individual soot particles to form a protein corona. While reduced aggregation may increase the availability of DEP for cellular uptake, it is important to note that this effect may also increase the potential toxicity of the particles by increasing their interactions with cells.

When comparing results from different collection methods, it was noted that PM samples collected with the CG-BioSampler induced more apoptosis than other PM samples. This might be related to some gaseous components and/or volatile and/or semivolatile organic compounds being collected by the CG-BioSampler, which may be lost during extraction of PM samples from filters (Wang et al., 2013). Moreover, the detected toxicity of the Dieselw sample was quite similar to the cell culture medium or water/DMSO mixture suspended samples. However, detected ROS responses were statistically significantly lower than Dieselm and Dieselw + w responses. Indeed, that response was at the same level as for Dieselw. This may indicate that the CG-BioSampler collection in water reduces the penetration of diesel particles into cells and, thus, diminishes the measured ROS response inside the cell. This could be tested in future studies by also collecting PM directly into cell culture medium.

For future research, it would therefore be relevant to explore toxicological responses to DEP collected by a CG-BioSampler using other biocompatible mediums. However, collection of DEP in cell culture medium may potentially involve other challenges. If collections are not carefully done and samples stored extremely well, there is a high possibility of microbial contamination, especially if samples are stored for long periods of time.

5 | CONCLUSIONS

The CG-BioSampler, as well as the DGI system, allow for high-efficiency PM collection for toxicity studies. However, the CG-BioSampler collection method seems to better preserve PM properties and may therefore be a more useful collection method for PM toxicity studies. This seemed to be evident, especially when comparing the Dieselw sample to the Dieselw sample. Even though these samples were in a similar medium, their toxicity differed significantly. It is also surprising that the Dieselw sample caused more apoptotic cell death than the other samples, but this may be related to differences in the collection method. The present toxicological results using A549 cells also suggest that the resuspension medium may strongly affect toxicological responses, at least when the PM samples are rich in organic compounds, for example, condensed on soot. Based on the current results, it was shown that resuspension of PM in pure water resulted in the lowest toxicological responses, indicating a low biological activity of PM and associated constituents. To conclude, the CG-BioSampler collection method of PM samples represents a competitive alternative to air filter sampling when assessing the toxicity of different PM in emissions and air pollution.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.
REFERENCES


SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

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